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**MYCOTOXIN (AFLATOXIN & OCHRATOXIN) AND PESTICIDE RESIDUES IN
CAPSICUM FRUTESCENTS IN PUNJAB, PAKISTAN**

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ABSTRACT

Natural (Mould growth) as well as man made contaminants (pesticides) decreases the quality of food, and also creates a potential risk for human. Pesticides, although banned still used in agriculture practices may lead serious health diseases. The study was planned to determine occurrence of mycotoxin (Aflatoxin & Ochratoxin) and pesticide residue in chillies commercially available in the five big city markets of Punjab, Pakistan. Thin Layer Chromatography (TLC) was used for the detection of mycotoxin and Densitometer was used for its quantification. Pesticide residues were quantified by High-Performance Liquid Chromatography. Among aflatoxin, B₁ was detected in 71% samples whereas 14% samples were detected positive for ochratoxin. Among pesticides, aldrin was found positive in 42% samples and Endosulfan was found in 24% samples. Contamination of Endosulfan and aldrin were higher as compared to DDT and DDE. Mycotoxin and Pesticide levels frequently exceed the limits in chillies and that risks exist for consumers.

Keywords: aflatoxin, ochratoxin, pesticides, chilli, Pakistan

INTRODUCTION

From global prospective of food safety and food security, mycotoxin contamination of foods has gained much attention as potential health hazards for humans and animals (Iqbal *et al.*, 2011). Cereals and other crops are exposed to fungal attack in the field or during storage and this attack may result in mycotoxin contamination of the crop. Ochratoxin A (OTA) and Aflatoxin B1 (AFB1) are among the most frequent observed combinations of mycotoxins in different plant products (Klarić *et al.*, 2013). It has been reported that 5-10% of agricultural products in the world are spoiled by mould contamination to the extent that they cannot be consumed by humans or animals.

Aflatoxins and Ochratoxin A are the most important for human health perspective and in developing countries such as Pakistan where climatic conditions favor the formation of these toxic metabolites (Qazi and Fayyaz 2006). The use of pesticides in agriculture is directly related to an increase in farm productivity. On one side, pesticides are very useful to increase harvest productivity, while on the other side, there could be a possible health risk to consumers (Conacher and Mes. 1993; Nieto *et al.*, 2009).

Considerations include health care and veterinary care costs, loss of human and

animal life, loss of forage crops and feeds, loss of livestock production, regulatory costs, and research cost focusing on relieving the impact and severity of the mycotoxin problem. It has been difficult to develop formulas for worldwide economic impact, therefore, reports on mycotoxin exposure or contamination are mostly deals single aspect of mycotoxins contamination (Hussein and Brasel 2001). Therefore, this study has been planned to check the contaminants (aflatoxin, ochratoxin & pesticides) status of commercial red chilli.

MATERIALS AND METHODS

Sampling

Twenty samples of red chilli (whole) were collected from local markets of five cities of Punjab (Lahore, Faisalabad, Multan, Sargodha, Rawalpindi), Pakistan. Due to uneven distribution of fungi in whole chillies, sample size was taken as 1.0 kg each as the sample also consumes for the analysis of pesticides residues.

Extraction of Aflatoxins, ochratoxin and pesticides from Chillies

Aflatoxin was extracted from chilli using methanol water mixture (80:20%, v/v) whereas ochratoxin was extracted by a mixture of ACN: H₂O (60:40; v/v). For the extraction of pesticides, 5% NaCl was added

in 25 gm chilli sample. After that 20% Anhydrous Sodium Sulphate and 70 ml Ethyl acetate was added into the flask. Placed the flask orbital shaker for 1 hour then filtered the sample through whattman filter paper. Evaporate the solvent in water bath and reconstitute it with 1 ml acetonitrile.

Detection of Aflatoxins

In the present study, Thin Layer Chromatography (TLC) was used for the detection of aflatoxin and Ochratoxin in chilli samples. Quantification of aflatoxin and Ochratoxin was carried out by using Scanner Densitometer.

TLC Conditions

The mixture of Chloroform: Acetone: water (88:12:1.5; v/v/v) was used as a developing solvent for aflatoxin and benzene–methanol–acetic acid mixture (18:1:1; v/v) was used for ochratoxin. TLC plates were checked under UV box (DESAGA, Germany) and positive samples were scanned under Shimadzu Dual Wavelength Flying Spot Scanning Densitometer for the quantification.

Pesticide analysis by HPLC

The analysis of pesticides was carried out on Shimadzu HPLC system 20A with quaternary pump and auto sampler. The Mobile phase was 100 % Methanol and flow rate was set at 0.5 ml/min. The Column used for analysis taken from Merck C₁₈ (250 x 4.6

mm), 5µm diameter. Detector Wavelength of 240 nm was used for the quantification of both samples and standard. A 20 µL of filtrate was injected to the system.

Statistical analysis

The data will be analyzed by applying suitable statistical technique (Chi-square test) and reported the results as mean ± SD (Steel et al. 1997).

Results

Thin Layer Chromatography (TLC) with Scanner Densitometer is a cheaper but reliable method for quantification of mycotoxin. Twenty samples of red chilli were collected from retail market of each city and analyzed. Contamination of aflatoxin (B₁, B₂, G₁ & G₂) in different cities of Punjab is shown in Table 1. It observed that chilli samples of Multan city were highly contaminated by Aflatoxin B₁ as compared to other cities. In Lahore city, it was found that 85% samples were contaminated by aflatoxin B₁ and presence of other aflatoxin types were comparatively less. Whereas in Faisalabad, Lahore, Rawalpindi and Sargodha, contamination of aflatoxin B₁ were, 70%, 60%, 75% and 65%, respectively.

The average value of AFB₁ calculated from Lahore and Faisalabad cities were 29.9 ppb (ranged from 8.5-70.5 ppb) and 28.35 ppb (6.8-55.8 ppb), respectively. The average

concentration of AFB₁ found in Multan, Sargodha and Rawalpindi cities were 33.86 ppb (8.4-77.5 ppb), 39.20 ppb (5.5-88.5 ppb) and 32.03 ppb (6.8-65.6 ppb), respectively. Overall, in all five cities, the average AFB₁ was 32.5 ppb. Aflatoxin B₂, G₁ and G₂ were also present in some samples but were below the permissible level of aflatoxin recommended by American and European countries.

Average concentrations of ochratoxin found in different cities are shown in Table 2. Maximum average conc. (8.57 ppb) of ochratoxin was found in chilli samples collected from Sargodha city between the range of 27.5-40.3 ppb. And the lowest average concentration (2.31 ppb) was found in Lahore City between the ranges of 20.7-25.4 ppb. Maximum number of samples (25) were detected by ochratoxin in Sargodha city and minimum number of samples detected in Lahore city. No sample was detected as positive in the samples of Rawalpindi city.

Organochlorine pesticides (Endosulfan, DDT, DDE and Aldrin) were checked in chilli samples by High Performance Liquid Chromatography (HPLC). Aldrin was found positive in 55% samples and DDT was found positive in 15% samples of red chilli.

In the current study we analyzed four members of organochlorine class

(Endosulfane, DDT, DDE and Aldrin). The highest concentration of Endosulfane was detected in Sargodha samples that were 9.0 ppb, shown in table 3. While in Faisalabad and Rawalpindi its level was 6.0 ppb and 7.0 ppb whereas in case of Multan samples its concentration was slightly low that was 2.0 ppb. There was no Endosulfane was detected in Lahore samples.

Similarly the concentration of DDT in Lahore samples was 3.0 ppb. Still the highest concentration of this pesticide was also detected in Rawalpindi samples that were 7.0 ppb. While in Faisalabad, Multan and Sargodha its level was 2.0 ppb, 3.0 ppb and 2.0 ppb respectively. Whereas the concentration of DDE in Faisalabad and Multan was 5.0 ppb and 1.0 ppb while in Sargodha it was 5.0 ppb and still Rawalpindi contained its highest value that was 7.0 ppb. Again no residue of DDE was detected in case of Lahore samples. On the other hand the highest level of Aldrin was estimated in Lahore samples that were 11.0 ppb. Minimum level was detected in Multan that was 5.0 ppb while almost same level was detected in cases of Faisalabad and Sargodha cities. In Rawalpindi its concentration was 10.0 ppb.

Table 1: Average concentration (ppb) of aflatoxin (B₁, B₂, G₁ & G₂) in different cities of Punjab

City	B1	B2	G1	G2
Lahore	29.10	12.52	5.74	2.34
Faisalabad	28.35	12.05	4.10	2.80
Multan	33.86	14.87	7.01	3.48
Sargodha	39.20	11.68	7.43	1.94
Rawalpindi	32.03	11.76	4.60	1.87

Table 2: Concentration (ppb) of Ochratoxin in red chilli (whole) collected from different cities of Punjab, Pakistan

Cities	Average Conc. (ppb)	± SD	Range (ppb)	Positive Samples (%)
Lahore	2.31	7.14	20.7-25.4	10
Faisalabad	4.22	11.07	10.6-37.4	15
Multan	7.34	15.60	21.6-44.6	20
Sargodha	8.57	15.39	27.5-40.3	25
Rawalpindi	ND	ND	ND	ND

Table 3: Average concentration of Pesticide (Endosulfan, DDT, DDE & Aldrin) residues in different cities of Punjab, Pakistan

City	Endosulfane	DDT	DDE	ALDRIN
Lahore	0.00	3.00	0.00	11.00
Faisalabad	6.00	2.00	5.00	8.00
Multan	2.00	3.00	1.00	5.00
Sargodha	9.00	2.00	5.00	8.00
Rawalpindi	7.00	7.00	7.00	10.00

DISCUSSION

Asia and Oceania cover a very large part of the globe, with most countries in the tropics and subtropics, so it is expected that most mycotoxin problems be caused by fungi, which grow at high temperatures (Pitt and Hocking 2003). Regulations for total aflatoxins dominate in food, whereas regulations for aflatoxin B₁ dominate in feed. Red chilli (whole) is highly vulnerable to fungal contamination and consequently mycotoxin formation due to their environmental (high humidity and high temperature) and processing conditions. Red chilli can be contaminated with toxigenic fungus in the field during crop production as

well as during drying process and instorage places.

Moisture (> 15%) is one of the factors which favour the growth of aflatoxin in food and feed samples. In the current study, the average moisture level in red chilli (whole) from marketed samples of different cities was 12.13 % which is not enough for the growth of aflatoxin. Miguel *et al.*, (1986) reported the 16.51% ± 0.45 minimal moisture content for growth and aflatoxin production by *Aspergillus parasiticus*. It was also reported that the best conditions for *Aspergillus* to grow on organic materials is when the temperature is warm and when the material has a high level of moisture 7% or more.

Red chilli (whole) samples from retail markets which were almost in dry form, however due to the poor storage conditions sometimes moisture level are exceeded from the recommended level due to which the fungal growth starts. We observed that in most of the seasons the moisture level is below than 12% and the toxin reported in this study was might be present in pre-harvesting conditions. Before selling in market, the chillies are dried first therefore; there are rare chances for aflatoxin growth during storage. The average value of AFB₁ calculated from Lahore and Faisalabad cities were 29.9 ppb (ranged from 8.5-70.5ppb) and 28.35 ppb (6.8-55.8 ppb), respectively. The average concentration of AFB₁ found in Multan, Sargodha and Rawalpindi cities were 33.86 ppb (8.4-77.5 ppb), 39.20 ppb (5.5-88.5 ppb) and 32.03 ppb (6.8-65.6 ppb), respectively. The maximum value allowed for spices according to the Turkish Food Codex is 5 µg/kg for AFB₁ and 10 µg/kg for total AF (Turkish Food Codex Regulation 2002). Overall, in all five cities, the average AFB₁ was 32.5 ppb. The average concentrations of AFB₂, AFG₁ & AFG₂ in 100 samples collected from five different cities of Punjab were 12.6 ppb, 5.8ppb & 2.5 ppb, respectively. Statistical analysis (Chi square test) showed that there are no

significant differences ($\chi^2 < \chi^2_{0.05}$) of aflatoxin occurrence among different cities. Similarly, Aflatoxin contamination was studied in different countries. AFB₁ was found in 9% of the fresh red pepper, 100% of the red-scaled pepper and 90.3% of the red pepper powder samples from the Gaziantep and Kahramanmara's regions. Aflatoxin B₁, B₂, G₁ and G₂ were determined in 32 red pepper samples collected from markets in the Istanbul region and was found that AFB₁ levels were higher than 5ppb in about 50% of the samples and total AF levels were >20 µg/kg in about 40% of the samples (Omurtag et al. 1998). Only aflatoxin B₁ was detected in 5 samples (14%) at levels between 10.5-31.2 µg/kg (Heperkan and Ermis, 2004). This was higher than the limits of the Turkish Codex. Forty four red-scaled pepper, 26 red pepper powder in Erzurum and 20 isot samples from Urfa were studied using TLC and found that 11.3% red-scaled pepper, 3.8% red pepper and 5% isot contained AFB₁ + G₁ + G₂ exceeded 5ppb (Erdogan, 2004). Hundred red pepper samples were examined in Istanbul, AFB₁ levels were found higher than the legal limits of the Turkish Food Codex (2002) and the European Commission (2002) (>5 µg/kg) in 18 (18%) red pepper samples. The highest AFB₁ level in the samples was 40.9 µg/kg

(Aydin *et al.*, 2006). In the current study it was found that almost 50% samples were contaminated by aflatoxin.

Chi square test showed that no significant differences ($\chi^2 < \chi^2_{0.05}$) of pesticides are occur among different different cities of Punjab). In normal circumstances an adult person takes almost 200 mg chilli in daily diet. So the average amount of Aflatoxin B₁ intake will be 6.65 ng/ day. While the amount of Ochratoxin will be 1.12ng. Similarly the amount of different pesticides (Endosulfane, DDT, DDE and Aldrin) get accumulated in adult human after getting an average of 200 mg chilli will be 10.28 ng, 0.06 ng, 0.24 ng and 6.87 ng/ day. Although their level was less than their respective permissible limits as mentioned in the permissible limit as this spice is use in different daily food items therefore, prolong use of such type of contaminated spice may cause serious health issues. Similarly Livestock meat and dairy products were the prime sources of human dietary exposure to organochlorines, since between 60-85% of the mean daily intakes arose from these particular food classes. These percentages are in accordance with the well documented fact that organochlorines predominantly accumulate in the lipid fractions of the human food chain, by which animal fatty foods have become a major

route of exposure for humans (Kannan *et al.*, 1992).

It has been observed that aflatoxin and pesticide levels frequently exceed the limits in red pepper (>5 µg/kg AFB₁ and 10ppb AF total; > 2mg/kg Pesticides) and that risks exist for consumers.

Conflict of Interest

Authors declare no conflict of interest

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