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Determination of Aflatoxins in Spices and Dried Fruits

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Abstract

The purpose of current study was to estimate the incidence of total aflatoxins (B_1+B_2+G_1+G_2) in unpack spices and dried fruits. A total of 90 samples included red chillies, black pepper, figs and dried apricots were picked from shops/markets situated in Lahore - Pakistan and were analyzed by using thin layer chromatography (TLC). The results showed that aflatoxin B_1 was detected in 24 (26%) samples. The results obtained were ranging between 23.99-97.42 μgkg^{-1} in spices, 47.68-75.78 μgkg^{-1} in black pepper, 6.72-14.43 μgkg^{-1} in figs while 13.2 μgkg^{-1} of aflatoxin B_1 was present in apricot sample. 39.28%, 18.18%, 5.0% and 40% samples of red chilli, black pepper, dried apricots and figs were found contaminated with aflatoxins respectively. Among contaminated samples 32.14% , 13.63%, 5.0% and 15% samples of red chilli, black pepper, dried apricots and figs, respectively were found contaminated with aflatoxins beyond permissible limits. Furthermore, 17.77% i.e. n=16 of the positive samples contained Aflatoxin B_1 level more than the permitted limit for entire aflatoxins as lay down by regulatory authority. From the study, it was concluded that a continuous and strict national monitoring plan is needed to improve quality and safety of spices and dried fruits supply in Pakistan.

Keywords: Aflatoxins; Contamination; Spices; Dried fruits; TLC.

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1. Introduction

Spices and dried fruits have become an essential part of food. Healthy life style needs provision of safe and good quality food. But unfortunately food can be easily contaminated through the environment. Harmful substances can gain entry in plants due to unpleasant external conditions. Plants naturally may have nontoxic and toxic substances in their various parts. Microorganisms are ubiquitously present in surroundings. Microorganisms can either be nonpoisonous or poisonous depending upon circumstances. Fungi are imperative plant pathogens which may cause production of so many malevolent

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metabolites [1]. Secondary metabolites named "Mycotoxins" are produced when filamentous fungus undergoes enzymatic or chemical reaction. These mycotoxins are very toxic and hazardous to animals, plants and human beings as well. Mycotoxin contamination causes huge damage to crops each year [2].

Aflatoxins are familiar mycotoxins which may badly affect all food entities. Warm and humid regions of the world are favorable environments for aflatoxins growth. According to International Agency for Research on Cancer, aflatoxins are included in class 1A human carcinogens [3]. Aflatoxin exposure may cause impaired growth, decrease immunity of body and its severity may cause liver cancer. Aflatoxin B_1 and B_2 are produced by *Aspergillus parasiticus* and *Aspergillus flavus* [4] while *Aspergillus parasiticus and Aspergillus nomius* produce aflatoxin G_1 and G_2 [5].

Contamination of aflatoxins in various food commodities is of prime concern as occurrence of aflatoxins a big threat to food security and quality. Aflatoxins contamination badly affect agriculture sector. Consequently, it may have bad impacts on main four pillars of food protection i.e. accessibility, availability, stability and utilization. Spices and dried fruits are more at danger to attack by aflatoxins due to unhygienic and bad storage conditions. Poor pre- and post-harvest conditions may cause contamination of spices and dried fruits. Careful drying, handling, transportation and storage are needed to avoid aflatoxin contamination in food commodities [6].

In one study, it was reported that spices imported from India were contaminated with aflatoxin B_1 within range of 1.2 – 968.3 μgkg^{-1}. World Health Organization set permissible levels for total aflatoxins in spices i.e. 30 μgkg^{-1} [7].

Aflatoxin contamination in various crops may result in economy losses as well as severe deterioration of human health. Scientists around the world are working on advanced technologies in order to reduce aflatoxins contamination during pre- and post-harvest conditions [8].

To investigate the current contamination scenario in Lahore, various samples of spices and dried fruits were checked for aflatoxin contamination.

2. Materials and Method

2.1. *Collection of samples*

For the present study, total 90 samples of black peppers *(Piper nigrum*), red chillies (*Capsicum annuum*), dried apricots *(Prunusarmeniaca)* and figs (*Ficuscarica*) were obtained from different shops/markets in Lahore like Barkat Market, BhattaChowk, Gharishaw Market, Ichhra Market, Jaffarabad Market, Market near Lahore Motorway, Samanabad, Township Market and shops at Wahdat Road.

2.2. *Reagents and chemicals*

Total Aflatoxins (B_1, B_2, G_1 and G2) standard were purchased from the Romer Lab, Singapore and TLC plates were purchased from Merck, USA. Acetone, acetonitrile

anhydrous ether, benzene, chloroform and other chemicals used for present work were of analytical grade.

2.3. *Grinding of sample*

In order to obtain homogenous samples AOAC method (number: 977.16) was used [9]. 500g of each sample was collected from market and carefully and uniformly mixed for 10-15 min. A representative sample for analysis was obtained by grinding whole sample in grinding mill. 50 g of every sample was used for AFs analysis in collected samples. All grinded samples were stored in air tight polyethylene bags.

2.4. *Aflatoxin extraction*

50 g of grinded sample was taken in 500 mL conical flask. 25mL of water, 25 g diatomaceous earth and 150 mL chloroform were added. The whole mixture in flask was mixed thoroughly for 30 min on wrist shaker. The mixture was filtered by using Whatmann No. 1 filter paper and 50 mL of mixture solution (chloroform) was obtained in a beaker. This sample solution was evaporated on water bath at 60 ºC [10].

2.5. *Thin Layer Chromatography (TLC)*

Spotting of sample 2.0, 3.5, 5.0, 6.5, 10, 15 and 25 µL was done on TLC plate (nearly 1.5 cm from the base) by using micro syringe. Similarly, spotting of 1, 2.5 and 5 µL standard was done. After spotting, the plate was paced in first tank containing anhydrous diethyl ether. After developing plate in first tank, the plate was dried and was placed in tank containing acetone-chloroform (1:9, v/v). Depending upon R_f values, the ratio adjustment was done. The plate was observed under UV light (365 nm) for checking the presence or absence of aflatoxins. The concentration of aflatoxins depends on the intensity of fluorescence. R_f values and sample color is analogous to aflatoxin standard.

2.6. *Detection and quantification*

Fluorescent intensities of standards spots and sample spots showing aflatoxins were compared. The amount of aflatoxins in spices and dried fruits samples was determined in $\mu g kg^{-1}$ using subsequent formula:

$$\text{Aflatoxins in } \mu g/kg = \frac{S \times Y \times V}{Z \times W}$$

Where,

S: Volume of aflatoxin standard in mL of correspondent intensity to Z = mL of sample
Y: Aflatoxin standard concentration in mg/mL
V: Volume required diluting final extract in mL
W: Effective weight in grams contained in final sample extract
Z: Volume of sample extract in mL required to confer fluorescence intensity analogous to that of S = mL of aflatoxins standard

The obtained results were analyzed statistically. Standard deviation, Mean, Max and Min were calculated.

3. Results and Discussion

3.1. *Screening of samples*

Different food entities were collected from Lahore city and were subjected to aflatoxins analysis by thin layer chromatography (TLC) for quantitative and qualitative analysis of aflatoxins. It was observed that 24 samples of spices and dried fruits showed Aflatoxin B_1 presence, however 66 samples were not contaminated with aflatoxins (Table1).

Table1.Screening of food samples for aflatoxin done by TLC.

AF	Sample	No. of Samples	No. of Contaminated Samples	No. of Non Contaminated Samples	Contamination (%)	Max μgkg^{-1}	Min μgkg^{-1}	Permissible limit
B_1	Figs	20	8	12	40%	14.43	6.72	10 μgkg^{-1} USFDA
	Apricot	20	1	19	5%	13.2	ND	10 μgkg^{-1} USFDA
	Black Pepper	22	4	18	18%	75.78	47.68	10 μgkg^{-1} USFDA
	Red Chili	28	11	17	39%	97.42	23.99	50 μgkg^{-1} [11]

*ND = Not Detected

Aflatoxin (AFB_1) was found in 24 samples collected from various areas of Lahore, Pakistan but other Aflatoxins B_2, G_1 and G_2 were absent in all collected food samples. Samples of red chillies, black pepper, figs and dried apricots were contaminated with aflatoxin B_1 within the range 6.72- 97.42 μgkg^{-1}.

Different tolerance limits for aflatoxins were set by USFDA (United State Food and Drug Administration), i.e. 10 μgkg^{-1} in dried fruits, black pepper and in further processed products [12]. 17 samples of red chillies were from aflatoxins contamination. 4 samples of ground black pepper contained aflatoxinsin the range of 47.68-75.78 μgkg^{-1} and among aflatoxin contaminated samples, 3 were found beyond permissible levels while 1 was within the range, as placed by UNCTAD (United Nation Conference on Trade and Development) for human consumption i.e. below 50 μgkg^{-1} for red chillies. According to UNCTAD, 1996, permissible level of aflatoxins in red chillies is 50 μgkg^{-1} [13].

17 samples of red chilli out of 28, 18 samples of black pepper out of 22, 19 samples of dried apricots out of 20 and 12 samples of figs out of 20 were found uncontaminated with aflatoxins. The maximum contamination beyond permissible limits i.e. 32.14% was found in red chillies as compared to other food commodities. However, overall % contamination of 39.28%, 18.18%, 5% and 40% were found in red chillies, black pepper,

dried apricots and figs respectively. Maximum number of figs was found contaminated with aflatoxins. The results of positive samples are given in Table 2.

Table 2. Positive samples were triplicated; results showed the concentration of AFB_1 contamination in red Chillies, black pepper, Figs and dried apricots.

Sample Number	1 Aflatoxin B_1 μgkg^{-1}	2 Aflatoxin B_1 μgkg^{-1}	3 Aflatoxin B_1 μgkg^{-1}	Aflatoxin B_1 Average ± SD μgkg^{-1}
Chilli A	68.08	68.87	68.34	68.43 ± 0.40
Chilli D	24.12	23.86	24.01	23.99 ± 0.13
Chilli G	39.04	39.55	39.87	39.49 ± 0.42
Chilli I	96.98	97.71	97.56	97.42 ± 0.38
Chilli K	63.51	63.91	63.03	63.48 ± 0.44
Chilli N	69.45	69.94	69.25	69.54 ± 0.35
Chilli O	89.56	89	89.05	89.20 ± 0.30
Chilli P	59.22	59.46	59.64	59.44 ± 0.21
Chilli R	76.45	77.44	76.98	76.95 ± 0.49
Chilli Z	71	70.95	71.45	71.13 ± 0.27
Chilli BB	77.89	78.03	77	77.64 ± 0.55
Pepper O	47.3	47.72	48.02	47.68 ± 0.36
Pepper T	76.01	75.73	75.6	75.78 ± 0.21
Pepper U	53.48	53.56	53.24	53.42 ± 0.16
Pepper V	63.89	64.93	64.5	64.44 ± 0.52
Fig A	11.56	11.89	11.2	11.55 ± 0.34
Fig B	13.56	13.93	13.01	13.51 ± 0.46
Fig E	14.32	14.73	14.25	14.43 ± 0.25
Fig F	9.6	9.59	9.35	9.51 ± 0.14
Fig K	9.58	9.29	9.78	9.55 ± 0.24
Fig L	8.34	8.63	8.78	8.58 ± 0.22
Fig S	9.68	9.26	9.43	9.45 ± 0.21
Fig T	6.87	6.85	6.45	6.72 ± 0.23
Apricot T	12.89	13.26	13.45	13.2 ± 0.28

The results in present study depicted that figs samples showed contamination ranging between 6.72-14.43 μgkg^{-1} and merely one sample of dried apricots was contaminated with aflatoxin B_1. Aflatoxin B_1 detected in sample of apricot was 13.2μgkg^{-1} exceeding the USFDA permissible levels. The range of aflatoxin contamination in red chillies was 23.99-97.42 μgkg^{-1}. 9 chili samples were exceeding permissible levels set by UNCTAD and 2 were within the permissible levels. 97.42μgkg^{-1} in red chili sample was the highest amount of aflatoxin which was beyond the set regulations. According to WHO (World Health Organization) the recommended aflatoxins level for red chilli is 30μgkg^{-1} [14]. As aflatoxins are toxic secondary metabolites so their detection in different food commodities like dried fruits and spices is very important [15]. The results are given in Fig. 1.

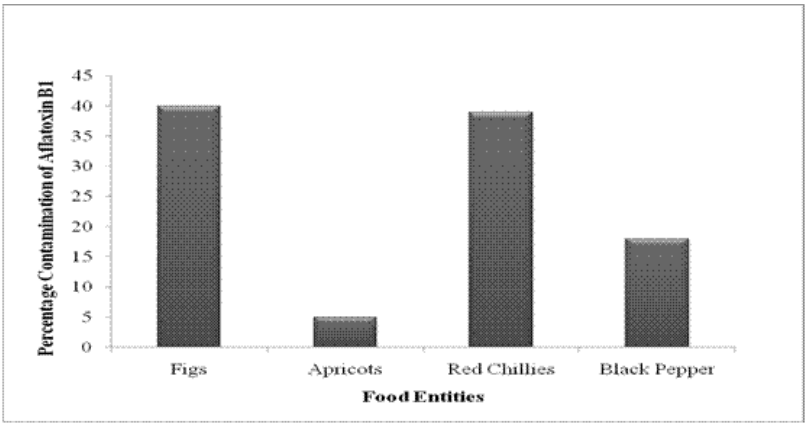

Fig. 1. Overall Aflatoxin contamination in different food commodities.

4. Conclusion

It was concluded from above study that the percentage of aflatoxin contamination in selected food commodities is low i.e. 26%. Only 24 out of 90 samples showed the presence of Aflatoxin B_1. But this is an alarming condition because regular intake of food contaminated with aflatoxins may cause cruel health damage to the consumers. As spices and dried fruits are vital parts of human diet and may badly affect by aflatoxins contamination; so these food commodities should be tackled with care in order to avoid aflatoxins contamination. The authorities in Pakistan should check and implement regulations strictly to monitor the food samples for aflatoxins contamination in food commodities on regular basis to save public health. There should be proper measures while harvesting, collecting, transporting, drying and storing food entities. It is suggested for consumers to buy spices, dried fruits and other food items from authentic and trustworthy retailers.

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