Aflatoxin Contamination of Spices Sold in Different Markets of Peshawar

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Summary: The present study was carried out to investigate the contamination level of total aflatoxin in locally available spices collected from three different locations of Peshawar. Eighteen samples of six different types of spices such as coriander, cumin seed, powdered red pepper, omum seed, black pepper and turmeric powder were analyzed for aflatoxin B_1, B_2, G_1 and G_2 by using thin layer chromatography. Out of 18, analyzed samples 12 samples were found contaminated with aflatoxin B_1 and. In the samples of hashtnagri coriander (5.59 µg / kg total aflatoxin) was found contaminated above the maximum level (4 µg / kg) prescribed by European Commission, while the other samples were either not contaminated or contaminated below the maximum level. Omum and turmeric were the samples contaminated (6.54 µg / kg) above the maximum level collected from firdos market. Similarly the samples collected from haji camp have coriander (6.54 µg / kg) and omum seed (7.46 µg / kg) contaminated above the maximum level. The overall results revealed that coriander and omum seed were found contaminated very much in all the samples analyzed for total aflatoxin.

Key words: Spices, total aflatoxin, contamination, Peshawar.

Introduction

Mycotoxins are naturally-occurring toxins produced by certain fungi that can grow on foods such as cereals, nuts, dried fruits, spices and legumes under certain environmental conditions. The most commonly observed mycotoxins include the aflatoxin (B_1, B_2, G_1 & G_2) and ochratoxin A. Aflatoxins have been shown to cause cancer of the liver in laboratory animals and to directly damage DNA. They are also considered to cause liver cancer in humans, particularly in a number of developing countries, where high levels of aflatoxins are found in some staple foods [1].

Fungi are the predominant contaminants of spices [2], but most such microbial populations are probably regarded as commensally residents on the plant. Soil and air is the main inoculums source for causing contamination in crude spices in field. Other practices like harvesting, handling and packing cause additional contamination. Moreover, spices are collected in tropical areas by simple methods and are commonly exposed to many contaminants before, being enough to prevent microbial growth. They are also stored in conditions favoring contamination by insects, rodents, and other vermin. Decontamination with ethylene oxide, irradiation, or other acceptable methods reduces considerably microorganisms present in spices [3].

Aflatoxins are a group of highly toxic secondary metabolic products of *Aspergillus flavus* and *Aspergillus parasiticus*, have carcinogenic and teratogenic effects to livestock and humans [4]. Aflatoxins in various agricultural products can be contaminated when drying of agricultural comm.-odities is delayed or moisture level exceeds critical values for the mold growth during storage of the crops. Especially, spices are usually produced in countries with tropical climates that have high temperature, humidity and rainfall [5]. These climatic conditions are favorable to aflatoxin contamination. In recent years the natural occurrence of aflatoxins in spices has been studied by several researchers [6, 7].

Being a product of agricultural practice, spices can carry high numbers of microorganisms as well as mycotoxins, especially aflatoxin B1. Thus, effective control of these parameters is a prerequisite for their utilization in the food sector [8]. Fungi are a normal component of food microflora and may be responsible for spoilage and production of mycotoxins [9-11]. Contamination of spices with aflatoxins can cause potential carcinogenic effects if absorbed even in small amounts [10].

Chili (*Capsicum annuum* L.) is one of the important crops of India. The use of cold stores for chili storage has now become almost a general practice among farmers. After harvest, the dried chilies were kept in cold stores as the produce fetches premium price due to excellent retention of the colour. During storage, chilies may be infected with molds. Many agricultural commodities such as cereals, oil seeds, spices, dry fruits and feeds have also been reported to be contaminated with the toxins produced by molds [12].

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The present study was thus designed to assess the aflatoxin (B_1, B_2, G_1 & G_2) levels in different samples of spices from different locations in Peshawar, Pakistan. The purpose of the present study was to assess the aflatoxin content in different spices. The information will be helpful for higher authorities to establish regulations and safe limits for this toxin present in spices.

Results and Discussion

In this study 18 samples of spices were analyzed for total aflatoxin. Out of which 12 samples were found contaminated with total aflatoxin in the range of 1.86 - 7.46 µg / kg. The result of the samples collected from Hashtnagri market is shown (Table-1). Coriander, omum seed and black pepper show aflatoxin production in which coriander (5.59 µg / kg) is above the maximum level (4 µg /kg) set by European Commission [13], for total aflatoxin. Red pepper, cumin seed and turmeric were not contaminated with mould and hence there is no aflatoxin production. The absence of aflatoxin in some of the samples most probably shows the unfavorable conditions for its development during production, product storage and distribution. The absence of aflatoxin is an important finding with respect to the safety of spices and their mixtures [8]. Good manufacturing practices after harvesting such as cleaning, drying, and packaging will minimize mould growth and aflatoxin production in spices. The mycological quality of some spices on the market, especially of pepper, is quite poor, bearing many genera and species of fungi. Most fungi are present on pepper of the post-harvest and storage type, which develop after harvest if relative humidity is not controlled during storage [9]. Samples of whole or ground black pepper from various sources yield numerous colonies of several species of *Aspergillus* [14].

Table-1: Aflatoxin analysis of spices collected from Haushtnagri Peshawar.

Sample	Aflatoxin (µg/kg)				
	B_1	B_2	G_1	G_2	Total
Coriander	ND	ND	5.59	ND	5.59
Red pepper	ND	ND	ND	ND	ND
Omum	ND	ND	2.49	ND	2.49
Cumin seed	ND	ND	ND	ND	ND
Turmeric	ND	ND	ND	ND	ND
Black pepper	3.73	ND	ND	ND	3.73

ND: Not detected

The results of the samples obtained from Firdos are presented (Table-2). Which range from 1.86 to 6.54 µg / kg for total aflatoxin. All the samples were contaminated except cumin seed. Omum seed and turmeric (6.54 µg / kg) were contaminated above the permissible level (4 µg / kg) set by European Commission [13], while the other spices were below that limit. The results of the spices collected from Haji Camp market are shown (Table-3).

Table-2: Aflatoxin analysis of spices collected from Firdos Peshawar.

Sample	Aflatoxin (µg/kg)				
	B_1	B_2	G_1	G_2	Total
Coriander	3.27	ND	ND	ND	3.27
Red pepper	2.18	ND	ND	ND	2.18
Omum	6.54	ND	ND	ND	6.54
Cumin seed	ND	ND	ND	ND	ND
Turmeric	6.54	ND	ND	ND	6.54
Black pepper	ND	ND	1.86	ND	1.86

ND: Not detected

Table-3: Aflatoxin analysis of spices collected from Haji Camp Peshawar.

Sample	Aflatoxin (µg/kg)				
	B_1	B_2	G_1	G_2	Total
Coriander	6.54	ND	ND	ND	6.54
Red pepper	3.27	ND	ND	ND	3.27
Omum	3.73	ND	3.73	ND	7.46
Cumin seed	2.18	ND	ND	ND	2.18
Turmeric	ND	ND	ND	ND	ND
Black pepper	ND	ND	ND	ND	ND

ND: Not detected

The most contaminated sample was omum seed (7.46 µg / kg total aflatoxin) which was contaminated with aflatoxin B_1 (3.73 µg / kg) as well as G_1 (3.73 µg / kg). Coriander is also contaminated above the maximum level (6.54 µg / kg total aflatoxin), while turmeric and black pepper did not show any contamination. Mostly spices are harvested in poor sanitary conditions. These improper conditions are convenient for the biosynthesis of aflatoxins. The results are also in accordance to the findings that the growing conditions, harvesting and processing methods, storage conditions and post harvest treatments should be carefully controlled in order to prevent aflatoxin risks due to contaminated spices [15].

Experimental

Collection of Samples

A total of 18 samples of different spices such as coriander, cumin seed, powdered red pepper, omum seed, black pepper and turmeric powder were purchased from three different locations/markets of Peshawar. All the samples were transferred to Mycotoxins Laboratory, Food Technology Centre, PCSIR Laboratories Complex Peshawar and were grounded by grinding mill for the purpose of obtaining a homogeneous and representative sample (100 g). These samples were stored in polyethylene bags until analyzed.

Analysis

Chemicals

All the chemicals of analytical grade used in the present study were procured from BDH (Poole, England), Merck (Darmstadt, Germany) and Sigma Chemicals (ST. Louis, USA). Standards of aflatoxin B_1 (2.02 µg / ml), aflatoxin B_2 (0.500 µg / ml), aflatoxin G_1 (2.01 µg/ ml) and aflatoxin G_2 (0.500 µg / ml) were purchased from Biopure (Tecknopark Tullin, Austria). Standard stock solutions of AFB_1, AFB_2, AFG_1 and AFG_2 of concentrations 1 µg/ ml each were prepared by diluting in benzene/ acetonitrile (98:2; v/v). These stock solutions were then stored at 4 °C in refrigerator, wrapped in aluminum foil due to that aflatoxins gradually breakdown under UV light.

Determination of Total Aflatoxins

Aflatoxins were determined according to standard method of Association of Official Analytical Chemists (AOAC), by thin layer chromatography [16]. Briefly, 50 gm test sample was extracted with 250 ml acetone/water (85:15 v/v) using blender for 3 minutes and filtered through whatman filter paper No 4. A 150 ml of filtrate was collected in 400 ml beaker. Then 170 ml of 0.02 N sod hydroxide and 30 ml ferric chloride along with about 3 gm basic copper carbonate added to the filtrate in 400 ml beaker, mixed well and added to the mixture in 600 ml beaker. This solution mixture was filtered and transferred 150 ml to 500 ml separating funnel. To this 150 ml of 0.03 % sulphuric acid was added and extracted twicely with 10 ml of chloroform. Lower chloroform extract layer was transferred to another separating funnel and 100 ml of 0.02 M potassium hydroxide was added, swirled gently for 30 seconds and left it for layer separation. Chloroform extract layer was collected in a vial. Of this 8 ml was evaporated to dryness at 45 °C under gentle stream of nitrogen on a heating block. The residue was dissolved in 200 µl benzene/ acetonitrile (98:2 v/v) and subjected to thin-layer chromatography.

Final identification and quantification of total aflatoxin were performed by one-dimensional TLC on pre coated silica gel plates (Merck, Germany). The plates were developed in a saturated chamber with chloroform/xylene/acetone (60:30:10; v/v/v). The samples spots were observed under long wave ultraviolet light (λ=365 nm) and determined by visual comparison with aflatoxins standard spots. Confirmation of the identity of aflatoxins was carried out with the spray of 50 % sulphuric acid and using the Trifluoroacetic acid (TFA) reaction [17].

Note

AFs are carcinogens and care should be exercised to avoid personal exposure and potential risk of contamination. All handling of pure compounds were done in the fume hood with protective gear such as safety glasses, gloves, laboratory coat and a disposable face mask. The glassware were washed with hypochlorite and dilute acid before re-using and the waste materials treated with hypochlorite before disposal.

Conclusion

It is concluded from the results that, some spices sold commercially in different markets of Peshawar are contaminated with aflatoxins at levels exceeding the permissible levels set by European commission. The aflatoxin analysis of the spices revealed mainly the presence of aflatoxin B_1 and G_1, which shows the possibility of fungal contamination during their production, marketing and storage. It is essential to investigate further the presence of other mycotoxins in these commodities. The data obtained will be of value in assessing the quality of foods and as an indicator of the potential for mycotoxin production.

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