Sci., Tech. and Dev., 32 (1): 16-23, 2013

REDUCTION OF AFLATOXINS IN DUNDI-CUT WHOLE RED CHILLIES *(CAPSICUM INDICUM)* BY MANUAL SORTING TECHNIQUE

MOBEEN AHMED KHAN*, MUHAMMAD ASIF ASGHAR, AFTAB AHMED, JAVED IQBAL AND ZUZZER ALI SHAMSUDDIN

Food and Feed Safety Laboratory, Food and Marine Resources Research Centre, PCSIR Laboratories Complex, Shahrah-e-Salimuzzaman Siddiqui, Off University Road, Karachi-75280, Pakistan.

Abstract

Dundi-cut whole red chillies *(Capsicum indicum)* are the most revenue-generating commodity of Pakistan. Accordingly, the competence and magnitude of manual hand-picked sorting of red chillies on the reduction of total aflatoxins (AFs) content were assessed during the present study. AFs contents were determined by thin layer chromatography (TLC) technique. On the basis of AFs content, red chilli samples were grouped as Group A with 1 to 20µg/kg, Group B with 20 to 30µg/kg, Group C with 30-100µg/kg and Group D quality samples with 100 to 150µg/kg. Physically identified defects including midget/dwarfed, damaged, broken, dusty and dirty were looked for and such pods were removed. A reduction of 90-100% of AFs was achieved in Group A, 65-80% in B, 65-75% in C and 70% in D quality samples. An average of 78% reduction in AFs content was achieved. Hence, the non-destructive physical hand-picked sorting of red chillies can be applied as a rapid, safe and cost effective method for the reduction of AFs content in red chillies with preserved nutritional values.

Keywords: Aflatoxins, Chillies, Reduction, Physical sorting, TLC.

1. Introduction

Red chillies (*capsicum indicum*) have gained a favourable admiration due to their use in blended spices as well as in almost all sorts of foods. Owing to their vital pharmacological activity, chillies are also used for their pungency in medicine (Reyes-Escogido et al., 2011). However, aflatoxin (AFs) contamination of red chillies has threatened the spice industry. Poor harvesting practices, improper drying, handling, packaging, storage and transport of chillies facilitate fungal growth and thus results in the increased risk of AFs contamination (Alim-un-Nisa et al., 2012). The fungal strains *Aspergillus flavus* and *Aspergillus parasiticus* are responsible for producing AFs under suitable conditions (Paterson, 2007). AFs are the carcinogenic, mutagenic and immunosuppressive fungal metabolites. To date, 18 different types of AFs have been identified. However, only four aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) are of considerable importance regarding their recurrence amongst cultures and food products (Reddy and Waliyar, 2012). AFs can create potential risk to human health with aflatoxicosis and cancer (Jeffrey and Williams, 2005).

The pre-harvested and stored products are prone to fungal attack. The insect wounds on the pistil of the flowers might serve as the germinating beds for fungal spores, thus, resulting in AFs production (Thanaboripat, 1988). Post-harvest contaminations of AFs take place particularly during floods, unseasonal rains or improper storage of insufficiently dried agricultural products. The temperate, sub-tropical and tropical climates invite aflatoxin-producing moulds (Cotty and Jaime-Garcia, 2007). For instance, Iqbal et al. (2011) reported that the AFs contamination in red chillies during the summer season was found higher than the winter season. Moreover, red chillies are prone to attack by a broad range of microbes due to adverse assortment situation like unpretentious production process, poor collection conditions, and

***Author for correspondence E-mail:mobeenaklivdetox@yahoo.com**

insufficient drying times (Banerjee and Sarkar, 2003; Cokosyler, 1999).

Spices sold in wholesale markets and *bazaars* in loose packaging could be easily infected through dust, waste water and animal/human excreta (Schweiggert et al., 2005). Dundi-cut whole red chillies are the most revenue generating commodity of Pakistan. Chillies are cultivated for domestic use and for export purposes as well. The growers/farmers of red chillies are facing problems regarding AFs contamination due to their drying in open fields and improper storage conditions.

It has been reported that chillies and chillies products are susceptible to mycotoxins contamination, as per surveys reported from UK (MacDonald and Castle, 1996), Spain (Santos et al., 2010), Australia (Klieber, 2000), Morocco (Zinedine et al., 2006), Turkey (Set and Erkmen, 2010) and Pakistan (Iqbal et al., 2010). In particular, AFs are of concern, as they are the most common mycotoxin found in chilli products around the world. Several countries, including Pakistan, have set forth the guidelines and acceptance level for AFs due to their frequent occurrence and toxicity. According to the European Commission Regulations (2010), the maximum acceptable level for AFB_1 and total AFs in red chilli is 5 and 10µg/kg, respectively. The tolerance level for total AFs in red chilli is 20µg/kg as per USA (FDA and FAO) recommendations (FDA, 2000) whereas acceptance level for total AFs in red chilli is 30µg/kg in Pakistan (PSQCA, 2009).

In the light of above mentioned facts, the use of effective and efficient physical and chemical methods is required for the reduction of AFs in red chillies. In addition to the technical and the economical considerations, such processes should reduce toxin concentrations to safe levels and in its own turn save the nutritive values of the red chillies.

Due to the severe toxicity associated with AFs contamination, considerable efforts have been done in the past to develop effective and reliable techniques for the reduction or elimination of AFs content. These include gamma radiation (Iqbal et al., 2012), storage at different climatic condition (Iqbal et al., 2011), dry heating, exposure to UV, hydrogen peroxide treatment and biological detoxification (Tripathi and Mishra, 2009). However, the efficacy of previously reported methods could be significantly improved in combination with physical sorting technique. The extent of physical sorting mainly depends on the physical nature of the chillies involved. The criteria for physical separation were based on degree of contamination, size weight after a final dust and dirt clearance. In this study, the effects of physical sorting (hand-picked) for the reduction of AFs are assessed in dundi-cut whole red chillies.

2. Materials and Methods

2.1 Sampling

In total, 70 batches (5kg each) of dundi-cut whole red chillies were collected from various agricultural farms located at Kunri-Sindh, Pakistan, during 2009 to 2010. It is well known that aflatoxins (AFs) are heterogeneously distributed throughout most food and feed commodities. For this reason, sampling procedure was based on the method as described in AOAC official method no. 977.16 to obtain the most accurate estimation of AFs (Trucksess, 2005). Samples were finally pulverised into particles ≤ 1 mm by passing through sieve No. 20 in a sample grinder (Cyclotec 1093 mill, Sweden) to obtain a homogeneous and representative sample.

2.2 Reagents

All the chemicals and solvents, used in this study, were of analytical grade procured from BDH (Poole, England), Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis-MO, USA). Crystalline AFs standards were purchased from Sigma-Aldrich (St. Louis-MO, USA). The crystals of AFs were diluted, using benzene-acetonitrile (98: 2 v/v) to obtain a concentration of 10µg/ml (stock solution). The concentration of the prepared solution was adjusted, using the spectrophotometer at a wave-length of the absorption maxima close to 350nm. The suitable concentration, used for thin layer chromatography (TLC), was 1.0µg/ml. Precoated TLC plates of Silica gel 60 (layer thickness 0.25mm, 20cm x 20cm) on glass or aluminium, without fluorescent indicator, were purchased from E. Merck (Dramstadt, Germany).

2.3 Physical Method for Chillies Sorting

Chillies samples were subjected to physical sorting to remove midget/dwarfed, damaged/

broken and discoloured (brownish grey) chillies. Furthermore, chillies off-standard by size (< 1.50cm) or weight (< 0.50g) were removed before finally dust and dirt. The method is as under:

2.3.1 Colour Sorting

In the first step, the discoloured (brownish grey) chillies were removed as compared to standard coloured (bright red) chillies. The colour sorting reduced the probability of having high AF contamination. The selected chillies were then preceded for size sorting.

2.3.2 Size Sorting

In the second step, the small chillies (< 1.50 cm) seemed to be liable for deterioration were removed. Moreover, the damaged, broken and the midget/dwarfed red chillies were also separated from the standard size chillies. The standard sized chillies were then prepared for the next step.

2.3. Weight Sorting

In the final step, those chillies were rejected which had weight lighter than 0.50g. Finally, dust and dirt were removed from the examined chillies batches.

2.4 Analysis of Aflatoxins

Chillies required rather rigorous clean-up procedure for removal of adulterants and interference resulting from various types of compounds for the analysis of AFs. A modified Romer method followed by bi-directional TLC was used for the detection of AFs (Shamsuddin et al., 1995). AFs were determined as described in the AOAC official method no. 975.36/968.22 (Trucksess, 2005), except that the defatting of the acetone extract of the sample was additionally performed with hexane (Shamsuddin et al., 1995). Furthermore, samples extracts were dissolved in benzene-acetonitrile (98:2 v/v). Finally, a spot of 2µl, 5µl and two spots of 10µl of samples and standards were applied on TLC plate. The plates were developed in unlined tank containing chloroform, xylene and acetone (6:3:1 v/v). TLC plates were observed under long wavelength UV light (254-366nm) in an enclosed viewing cabinet and samples were quantified by visual comparison with AFs standards.

The observed R_f values for standards AFs were AFB_1 = 0.71, AFB_2 = 0.64, AFG_1 = 0.61 and AFG_2 = 0.52. All positive red-chilli samples showed similar peaks with identical spectra as that of the standards. Furthermore, the presence of AFB_1 was confirmed by spraying the TLC plates with H_2SO_4 (50% v/v) and making the derivative with trifluoroacetic acid. Limit of detection (LOD) for AFB_1 and AFG_1 was 1µg/kg and for AFB_2 and AFG_2 was 0.50µg/kg, respectively. AFs were analysed in the batches: (a) prior to the chillies sorting and (b) after the chillies sorting process. The analysis was carried out in triplicate. The method was ISO/IEC-17025 accredited and dually certified by national (Pakistan National Accreditation Council, PNAC) and international (Norwegian Accreditation, NA) accreditation bodies.

3. Results and Discussion

Dundi-cut whole red chillies samples were analysed and the results are shown in Table-1.

Table 1. Effects of physical sorting on total aflatoxins ($B_1 + B_2 + G_1$ and G_2) content in dundi-cut whole red chillies

*Red Chilli Category	No. of samples	Total aflatoxins (B_1+B_2+G_1 and G_2) content in dundi-cut whole red chillies (µg/kg)				% Aflatoxins Reduction
		Before sorting		After sorting		
		Range	Mean	Range	Mean	
A	22	6.62-19.76	14.03 ± 4.23	1.17-1.88	0.47 ± 0.73	90-100
B	14	20.75-29.64	25.46 ± 3.32	3.96-9.95	7.61 ± 2.33	65-80
C	27	33.59-93.91	63.65 ± 16.57	10.96-23.54	15.72 ± 3.64	65-75
D	7	101.78-148.75	118.56 ± 20.92	30.3-43.85	34.70 ± 6.29	70

*** Red chilli samples were categorised into four groups (A, B, C and D) on the basis of their total aflatoxins (B_1+B_2+G_1 and G_2) content.**

All the contaminated samples were found to contain various levels of aflatoxins (6.62-148.75 µg/kg). The samples were sub-divided into four categories as regard to the AFs content in group A (22 samples) with 1 to 20 µg/kg, B (14 samples) with 20 to 30µg/kg, C (27 samples) with 30 to 100µg/kg and D (7 samples) with 100 to 150µg/kg. A reduction of 90-100% of AFs was achieved in category A quality samples, 65-80% in B quality, 65-75% in C and 70% in D' quality. An average of 78% reduction in AFs content was achieved.

The physical appearance of dundi-cut whole red chillies before (A) and after physical sorting (B and C) is represented in Fig. 1.

A: Before Sorting **B: After Sorting** **C: After Sorting**

Fig. 1. Dundi-cut whole red chillies before and after physical sorting/separation. A: Mix quality chillies (before sorting), B: Good quality chillies (after sorting) and C: Bad quality chillies (after sorting).

Before physical sorting, AFs level in maximum chillies samples were much above the European Union (EU) permissible limits, i.e., 10µg/kg. The findings of this study indicate that physical sorting would help to some extent and reduce the AFs content of chillies. After physical sorting, categories 'A' and 'B' were found fit for human consumption as per EU regulation while chillies under category 'C' were found fit for human consumption as per the rules set up by bodies in USA (FDA and FAO) and Pakistan (PSQCA). However, chillies under category D exceeded the limits of above mentioned regulatory agencies. Other methods, like, the use of binders, microwave treatment and UV etc., could also be used and count amongst the remedial options.

The colour sorting might help in reducing the AFs contamination in chillies. By size sorting, it was observed that the red chillies classified as small seemed to be liable for deterioration. Removal of small pods had a small impact in the reduction of AFs content. However, significant reduction in AFs contamination was achieved by the sorting of the damaged, broken and the midget/dwarfed red chillies. In principle, physical sorting reduces fungal deterioration. Consequently, red chillies with reduced AFs content were achieved.

This method has been practically demonstrated at Kunri, where the labour is economical and cheap for the physical sorting/ grading process. Kunri is a small town, which is located in the Sindh province, in the southern part of Pakistan (SBI, 2010). Similar practice has been done in UK, where UK Food Standard Agency (2005) suggested removing 4.92% spice samples, which were found beyond the legal limits of EU.

As far as, the quality improvement of the process applied to the red chillies is concerned, it would be better to commercialise red chillies (whole) with completely intact shell. The disfigured chillies could be visually segregated, as the consumers avoid eating them. This may be the first study carried out in Pakistan, regarding the control of AFs via the physical sorting of red chillies**.** The whole chillies are safer to AFs contamination as compared to crushed and powdered as they can be sorted physically as described above. Tripathi and Mishra (2009) reported that direct oven heating at 120°C for 12 hours resulted in the maximum 83.32% reduction of AFB_1 in chilli powder. Exposure to UV for one hour reduced AFB_1 about 59.62% while 58.32%

decline in AFB_1 was attained by hydrogen peroxide treatment. However, 66.2% reduction in AFB_1 has been achieved by biological detoxification.

The physical sorting methods are more efficient over other methods for AFs control. AFs are quite stable with their melting point of 268°C (O'Neil et al., 2001). In these circumstances, dry heating is not particularly effective. Prolonged heating may adversely affect the quality of the protein and availability of lysine (Mao et al., 1993). In physical sorting, no heating is required and, therefore, may not adversely affect the quality of the protein and availability of lysine. This effect has been practically demonstrated during the present study where about 78% reduction in AFs content was achieved. The physical sorting could reduce the AFs concentrations to safe levels and eliminate the sources responsible for the production of AFs. Nevertheless, the physical sorting not only reduces the AFs content but also save the nutritional values alongwith the organoleptic properties of the red chillies. This technique has already disseminated awareness during the monitoring of the strategic plans at the premises of the desired farmers, growers and stock holders. It is suggested that strategies should be taken right from the field where commodities grow, harvest and store.

The physical sorting as regards colour, size and shape significantly reduced the AFs levels in peanuts (Scussel and Mello, 2009). Furthermore, separation of heavily damaged ears reduced AF levels in maize as well (Sétamou et al., 1998). Sorting of physically damaged and infected grains (known from colorations, odd shapes and size) from the infected commodities can result in 40-80% reduction in AFs level (Park, 2002). Furthermore, Galvez et al. (2003) reported that the manual sorting of raw materials with initially high AFs contents (300 µg/kg) resulted in AFs-free peanuts. The results achieved during the present study were found in good correlation with the observations reported above.

To strengthen the exportable segment of red chillies in Pakistan, essential measures regarding the procurement, drying, handling and packaging practices alongwith appropriate storage and transport conditions, etc., have been suggested for the prevention and control of mycotoxins, particularly the AFs.

Controlling mold growth and mycotoxin production is of prime importance for the chillies and chilli's products holders. The task could be achieved by keeping moisture level as low as possible in order to discourage the mold growth and to keep the chillies fresh and safe enough for the marketable segment of Pakistan. Moreover, aeration of bins is of vital importance to reduce moisture migration and to keep dry enough the chilli and chilli stuffs.

The moist and humid climate (up to 8%) is supposed to be an ideal and safe to avoid fungal growth whilst humidity beyond 11% leads to fungal attack. At field priority should be given to discard diseased fruit that must be processed immediately after harvest and should be stored below 13°C. Moreover, powdered products should be packed right away below low moisture conditions and kept in sealed containers (Klieber, 2000). Owing to international economic and toxicological importance of mycotoxins, specific regulations have been enforced for red chilli trade. Forinstance, Choudhary and Kumari (2010) described different measures to minimise the risk associated with mycotoxins. In addition, Hell and Mutegi (2011) prescribed the AFs management via environmental control. As per Spices Board of India (2012) different factors, like, raw material, processing practices, packing, storing and transportation might affect the quality of the product. Care must be taken right from the pre-harvest operation upto delivering the product to the consumer desk. In addition, Organization of American States and Mayan Reserve Foundation jointly describe the different remedial measures to reduce AFs contamination in chillies, such as, storage at low relative humidity and temperature, use of shorten drying time and rapid delivering to the end user. The personnel involved in the process must be educated (Fact Sheet, 2004-2005).

The Food and Agriculture Organization (FAO) (2001) prepared a manual on the application of the Hazardous Analysis and Critical Control Point (HACCP) system for the prevention and control of mycotoxins. The food industries and official food control authorities effectively applied the HACCP system to prevent and control the threat related to possible

contamination of food products with pathogenic micro-organisms and chemical toxicants. The manual provides guidance to those industries/ institutions who obey the HACCP approach as regards of mycotoxin prevention and control (FAO/IAEA., 2001). It is earnestly required to develop and implement new and efficient physical methodologies that have become extremely essential for flourishing the chilli trade in Pakistan.

4. Conclusion

The results of this study demonstrate that the physical sorting regarding colour, size and shape can be used to reduce the AFs contamination of chilli offered for human consumption. Physical sorting can be successfully applied as a rapid, safe and cost effective solution to the problem in conjunction with non-destructive to the nutritional values, however, complete elimination of AFs is not possible. In order to prevent the hazards associated with the fungal contamination, number of procedures, such as, controlling moisture and temperature, packing, storage, etc., can reduce AFs from chillies and chillies products.

References

Alim-un-Nisa, N. Zahra, S. Firdous, N. Ejaz and S. Hina. 2012. Detection of aflatoxins in various samples of red chilli. *Pakistan Journal of Scientific and Industrial Research Series B: Biological Sciences*. 55(1): 27-29.

Banerjee, M. and P.K. Sarkar. 2003. Microbiological quality of some retail spices in India. *Food Research International*, 36:469-474.

Choudhary, A.K. and P. Kumari. 2010. Management of mycotoxin contamination in preharvest and post harvest crops: present status and future prospects. *Journal of Phytology*, 2: 37-52.

Cokosyler, N. 1999. Farkh yontemlerle kurutulan kirmizi biberlerde *Aspergillus flavus* gelisimi ve aflatoksin olusumunun incelenmesi. *Gida*, 24:297-306.

Cotty, P.J. and R. Jaime-Garcia. 2007. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *International Journal of Food Microbiology*, 119: 109–115.

European Commission Regulation (ECR). 2010. (EC No. 165/2010 of 26 February 2010) Amending regulation (EC) No 1881/2006. Setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Official Journal of the European Union*, 50: 8-12.

Fact Sheet. 2004-2005. Chillies & the aflatoxin contamination. Organization of American States in cooperation with the Mayan Reserve Foundation.http://www.tsdfbelize.org/OAS%20website/pics%20z_oasfactsheet/0201Aflatoxin.pdf..

FAO/IAEA. 2001. Manual on the application of the HACCP system in mycotoxin prevention and control. Training and reference centre for food and pesticide control. *FAO Food and Nutrition paper 73*, FAO, Rome, Italy.

FDA, U.S. Food and Drug Administration. 2000. Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed. http://www.fda.gov.

Galvez, F.C., M.L. Francisco, B.J. Villarino, A.O. Lustre and A.V. Resurreccion. 2003. Manual sorting to eliminate aflatoxin from peanuts. *Journal of Food Protection*, 66: 1879–84.

Hell, K. and C. Mutegi. 2011. Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. *African Journal of Microbiology Research*, 5:459-466.

Iqbal, Q., M. Amjad, M.R. Asi and A. Arino. 2012. Mold and Aflatoxin Reduction by Gamma Radiation of Packed Hot Peppers and Their Evolution during Storage. *Journal of Food Protection*, 75: 1528–1531.

Iqbal, Q., M. Amjad, M.R. Asi and A. Arino. 2011. Assessment of Hot Peppers for Aflatoxin and Mold Proliferation during Storage. *Journal of Food Protection*, 74: 830–835.

Iqbal, S.Z., R.R. Paterson, I.A. Bhatti and M.R. Asi. 2011. Comparing aflatoxin contamination in chillies from Punjab. Pakistan produced in summer and winter. *Mycotoxin Research*, 27 (2): 75-80.

Iqbal, S.Z., R.R. Paterson, I.A. Bhatti, M.R. Asi, M.A. Sheikh and H.N. Bhatti. 2010. Aflatoxin B_1 in chillies from the

Punjab region, Pakistan. *Mycotoxin Research*, 26 (3(:205-209.

Jeffrey, A.M. and G.M. Williams. 2005. Risk Assessment of DNA-reactive Carcinogens in Food. Toxicology and Applied Pharmacology, 207:628-635.

Klieber, A. 2000. *Chilli spice production in Australia*. A report for the rural industries research and development corporation. University of Adeleide.

Llewellyn, G.C., R.L. Mooney, T.F. Cheatle and B. Flanningan. 1990. Mycotoxin contamination of spices – an update. *International Biodeterior and Biodegrad*, 29:111-121.

MacDonald, S. and L. Castle. 1996. A UK retail survey of aflatoxins in 308 herbs and spices and their fate during cooking. Food Additive and Contaminants, 13: 121–128.

Mao, L-C., K.H. Lee and H.F. Erbersdobler. 1993. Effects of heat treatment on lysine in soya protein. *Journal of the Science of Food and Agriculture*, 62: 307–309.

O'Neil, M.J., A. Smith and P.E. Heckelman. 2001. *The Merck Index, An Encyclopaedia of Chemicals, Drugs, and Biologicals*, 13th Ed. Whitehouse Station, NJ, Merck & Co.

Pakistan Standard & Quality Control Authority (PSQCA). 2009. Standard Development Centre, Agriculture and Food Division.

Park, D.L. 2002. Effect of processing on aflatoxin. *Advances in Experimental Medicine and Biology*, 504: 173–179.

Paterson, R.R.M., 2007. Aflatoxins contamination in chilli samples from Pakistan. *Food Control*, 18: 817–820.

Reddy, S.V. and F. Waliyar. 2012. Properties of Aflatoxin and it Producing Fungi. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). http://www.icrisat.org/aflatoxin/aflatoxin.asp. [June 18, 2012].

Reyes-Escogido, M.L., E.G. Gonzalez-Mondragon and E. Vazquez-Tzompantzi. 2011. Chemical and Pharmacological Aspects of Capsaicin. *Molecules*,16: 1253-1270.

Santos, L., V. Marín, Sanchis and A.J. Ramos. 2010. Co-occurrence of aflatoxins, ochratoxin A and zearalenone in Capsicum powder samples available on the Spanish market. *Food Chemistry*, 122:26–830.

Schweiggert, U., K. Mix, A. Schieber and R. Carle. 2005. An innovative process for the production of spices through immediate thermal treatment of the plant material. *Innovative Food Science & Emerging Technologies*, 6:143-153.

Scussel, V.A. and F.R. Mello. 2009. Development of physical and optical methods for In-shell Brazil nuts sorting and aflatoxin reduction. *Journal of Agricultural Science*, 1: 3–14.

Sétamou, M., K.F. Cardwell, F. Schulthess and K. Hell. 1998. Effect of insect damage to maize ears, with special reference to Mussidianigrivenella (Lepidoptera: Pyralidae), on Aspergillusflavus (Deuteromycetes: Monoliales) infection and aflatoxin production in maize before harvest in the republic of Benin. *Journal of Economic Entomology*. 91: 433–438.

Set, E. and O. Erkmen. 2010. The aflatoxin contamination of ground red pepper and pistachio nuts sold in Turkey. *Food and Chemical Toxicology*, 48: 2532- 2537.

Shamsuddin, Z.A., M.K. Khan, B.A. Khan, M.A. Ahmad and A. Ahmed. 1995. Contamination of Red Chilli with Aflatoxin B_1 in Pakistan. *Mycotoxin Research*, 11:21-24.

Sindh Board of Investment (SBI), Government of Sindh 2010. Red chilli de-hydration plant – Kunri, Sindh. 2010. www.sbi.gos.pk/pdf/Red-Chilli-De-Hydration-Plant.pdf.

Spices Board of India, Ministry of Commerce and Industry. 2012. Guidelines on quality improvement.http://www.indianspices.com/html/qltyStandrdGuid.html[April 2, 2012].

Thanaboripat, D. 1988. Aflatoxin in spore of *Aspergillusflavus*. *Asean Food Journal*, 4:71-72.

Tripathi, S. and H.N. Mishra. 2009. Studies on the efficacy of physical, chemical and biological aflatoxin B_1 detoxification approaches in red chilli powder. *International Journal of Food Safety, Nutrition* and *Public Health*, 2:69-77.

Trucksess, M.W. 2005. Natural Toxins. In: W. Horwitz, G.W. Latimer (Eds.), *Official*

Methods of Analysis of AOAC International, AOAC International, Gaithersburg, MD, USA, pp. 1-85.

UK Food Standard Agency. 2005. Survey of spices for aflatoxins and ochratoxin A. Food Survey Information Sheet 73/05.

Zinedine, A., C. Brera, S. Elakhdari, C. Catano, F. Debegnach, S. Angelini, M. Faid, M. Benlemlih, V. Riardi and M. Miraglia. 2006. Natural occurrance of mycotoxins in cereals and spices commercialized in Morocco. *Food Control*, 17:868-874.