Journal of Biosphere, 2(1): 10-12, 2013 ISSN 2278 – 3342

Aflatoxin B_1 and B_2 contamination in red chilli powder sold loose in the markets of Jammu and Kashmir.

Shallu Samyal and Geeta Sumbali

Abstract: Aflatoxins are the most potent natural carcinogen known. Twenty one samples of red chilli powder sold loose in the markets were screened for the presence of aflatoxin ($B_1$and B_2) contamination. 19.0 per cent samples of red chilli powder sold loose were contaminated with aflatoxin B_1 whereas, 29.0 per cent samples were found to be positive for afla B_2. Detection of this mycotoxin in the market samples of red chilli powder from Jammu is a serious issue as it can adversely affect the health of consumers.

Key words: Aflotoxins; carcinogen; Jammu; red chilli.

1. Introduction

Chilli/red pepper belongs to the nightshade family Solanaceae and is a fruit of the genus *Capsicum*. Red chilli powder is world's renowned spice that is used in many cuisines and recipes of various cultures to add tangy taste to them. In addition, chillies are an important source of vitamins A, C and E for the world population (Bosland and Votava, 2000). They are powerful antioxidant and anti inflammatory agents containing high amounts of carotenoid that has provitamin A activity (Howard et al., 1994; Hornero-mendez et al., 2002). The ascorbic acid (vitamin C) and tocopherol (vitamin E) content in chillies is quite high, which makes them very effective as immune system stimulants and healing agents especially for cellular damage (Marin et al., 2004; Biacs et al., 1992).

Like other agricultural products, spices including red chillies may be exposed to a wide range of microbial contaminants, during pre and post-harvest periods (Koci-Tanackov et al., 2007). Although used in small quantities, dried spices are recognized as significant carriers of microbial contamination, primarily the xerophilic storage moulds and some bacteria (Romagnoli et al., 2007). Moulds are especially important because they drastically reduce the quality of food and create a potential risk for human health with the production of toxic metabolites known as mycotoxins. Owing to conditions of production and to poor storage conditions, products derived from *Capsicum* are susceptible to fungal contamination (Adegoke et al., 1995; Schweiggert et al., 2005). However, when these red chillies are dried to a satisfactory degree before storage, they may develop local pockets of fungal growth, many of which may consist of toxigenic strains. *Aspergillus* is the most commonly found filamentous moulds found in *Capsicum* powder. These moulds cause food spoilage, but they are also able to able to produce different mycotoxins.

Shallu Samyal(✉)
Department of Botany,
Govt. College for Women,
Parade, Jammu- 180001, J&K, India.
Email: shallusamyal@gmail.com

Geeta Sumbali
Department of Botany,
University of Jammu,
Jammu- 180006, J&K, India.

2. Materials and methods

2.1 Extraction of mycotoxins from red chilli powder

Domestic and market samples of chilli powder that were collected from Jammu and Kashmir division were analyzed for aflatoxin contamination by using modified multimycotoxin method developed by Roberts and Patterson (1975). In this method, 25g of the sample was taken in an Erlenmeyer flask containing 100 ml mixture of acetonitrile and 4% potassium chloride (90:10v/v). Extraction was done by putting the flask on a horizontal mechanical shaker for 30 minutes. Thereafter, extract was filtered through Whatman no. 41 filter paper. The filtrate was taken in 250 ml separating funnel, defatted and extracted twice with 50 ml iso-octane. The upper iso-octane layer containing lipid was discarded and 12.5 ml distilled water was added to the lower acetonitrile layer. Basic mycotoxins were extracted from the lower acetonitrile layer, thrice by using 20 ml chloroform each time. Lower chloroform acetonitrile layer was collected in a conical flask and drained through Whatman no. 41 filter paper having a bed of anhydrous sodium sulphate. The extract was collected in a beaker and marked as extract I. The aqueous layer left in the separating funnel was acidified with 1ml of 1.0 N HCl and the acidic mycotoxins were extracted from it thrice by using 10 ml chloroform each time. Lower chloroform layers were combined, passed through anhydrous sodium sulphate bed, collected in a beaker and marked as extract II. Extracts I and II were combined and then evaporated to dryness on a water bath.

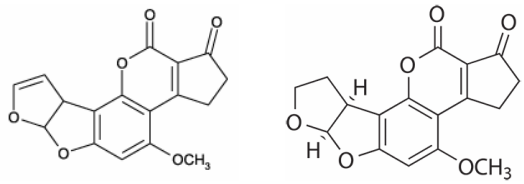
For pigment separation, dried residue was re-dissolved in 1.25 ml of acetonitrile and transferred into a dialysis sac made from dialysis tubing, which was thoroughly washed with distilled water. The dialysis sac was equilibrated against 25 ml of acetone water mixture (30:70 v/v) in a stoppered conical flask for 16 hours by gentle shaking on a wrist action shaker. To improve recovery of aflatoxins, dialysis sac was again equilibrated for 6 hours against 25 ml of acetone water mixture (30:70 v/v). Aqueous acetone dilysates were combined and extracted with 15 ml of chloroform three times in a separating funnel. Methanol (3 ml) was added to it for the clear separation of layers. Chloroform extracts were combined, passed through anhydrous sodium sulphate bed and dried on a water bath. Dried residue was dissolved in 1 ml of chloroform and stored in a small screw cap vial for detection of aflatoxins.

2.2 Preparation of TLC plates

Glass plates of 20 x 10 cm size were thoroughly cleaned with detergent, air dried and then cleaned with acetone soaked cotton to remove all grease. Silica gel-GF 254 (TLC grade) and distilled water (1:2 v/v) was taken in a stoppered round bottom flask and shaken thoroughly for 2 minutes to make a uniform slurry. This slurry was applied on glass plates with the help of an applicator so as to get a uniform thickness of 0.25cm. The coated plates were allowed to air dry for 2 hours and then activated in a hot air oven at 110ºC for 1 hour. These plates were then cooled to room temperature and used for thin layer chromatography.

2.3 Estimation of aflatoxins

For qualitative estimation of aflatoxins, known amount of sample extracts (50 µl) were applied with the help of micropipette on the activated TLC plates. The aflatoxin standards (B_1 and B_2) were also spotted on the TLC plates as reference spots and then developed with solvent system consisting of toluene: isoamyl alcohol: methanol (90:32:2 v/v). Developed TLC plates were examined under long wave UV light (365nm) and various spots of aflatoxins were located and marked with a sharp needle after comparing their fluorescence colour and Rf value with the standard spots. Chemical confirmation of aflatoxins was done by spraying 0.25% H_2SO_4, which changed the blue fluorescent spots to yellow (Stack and Pohland, 1975).



AFB_1 ($C_{17}H_{12}O_6$) AFB_2 ($C_{17}H_{14}O_6$)

Molecular structure of $AFB_1$and AFB_2.

3. Results and Discussion

During the present investigation, twenty one samples of red chilli powder sold loose in the markets of J&K were screened for the presence of $AFB_1$and AFB_2 contamination. Perusal of data presented in table 1 shows that 19.0 percent of the market samples that were sold loose were detected to be contaminated with aflatoxin B_1 whereas more samples (29.0 percent) were positive for afla B_2 contamination.

Table 1. Presence of aflatoxin in red chilli

Sample Code No.	AFB_1	AFB_2
L-1	-	-
L-2	+	-
L-3	+	+
L-4	-	+
L-5	-	-
L-6	-	-
L-7	-	+
L-8	-	+
L-9	-	-
L-10	-	+
L-11	-	+
L-12	-	-
L-13	+	-
L-14	-	-
L-15	-	-
L-16	-	-
L-17	+	-
L-18	-	-
L-19	-	-
L-20	-	-
L-21	-	-
No. of +ve samples	**4**	**6**
%. of +ve samples	**19.0**	**29.0**

- Not detected

Survey of literature shows that chilli powder samples have earlier also been detected to be contaminated with aflatoxins (Abdulkadir et al., 2002; Fazekas et al., 2005; Ozbey and Kabak, 2012). *Aspergillus flavus* and *A. parasiticus* are the most important producers of aflatoxins in various agricultural commodities (Cotty and Garcia, 2007). Aflatoxins are the most potent of all the mycotoxins, as they are known to have hepatotoxic, carcinogenic, teratogenic and mutagenic effects (WHO, 1979; IARC, 1993). Human foods are allowed 4-30 ppb aflatoxins, depending on the country involved (FDA, 2004). European Union, has imposed a maximum acceptable limit of 4 µg/kg in food for human consumption, the strictest in standard worldwide (EC, 2006), whereas Indian government under 57A of prevention of Food Adulteration Rules has imposed maximum permissible limit of 30 ppb for aflatoxin B_1 in all the foods prone to aflatoxin contamination (Sinha,1995). It has been estimated that more than 5

billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Strosnider et al. 2006). Post-harvest conditions such as storage, transportation and food processing amongst others; have been implicated as predisposing factors that enables *A. flavus* and *A. parasiticus* to produce aflatoxins in contaminated food (Wu and Khlangwiset 2010). In fact aflatoxin B_1 is the most toxic member of the group and its tolerance level has been fixed by World Health Organisation (1979) as 20 ppb. Detection of this toxin in chilli powder sold loose in J&K markets is a serious issue as it can adversely affect the health of consumers. Therefore, its management is urgently required.

References

Abdulkadir, E.E., Tahiya, A.A., Saif, N.A and Charles, S.B. 2002. Fungi and aflatoxins associated with spices in the Sultanate of Oman. *Mycopathol*. 155: 155-160.

Adegoke, G.O., Allamu, A.E., Akingbala, J.O. and Akanni, A.O. 1995. Influence of sun-drying on the chemical composition, aflatoxin content and fungal counts of two pepper varieties. *Capsicum annum* and *Capsicum frutescens*. Plant Food Human Nutr. 49: 113-117.

Biacs, P., Czinkotai, B. and Hoschke, A. 1992. Factors affecting stability of colored substances in paprika powders. *Jour. Agric. Food Chem*. 40: 363-367.

Bosland, P.W. and Votava, E.J. 2000. Peppers: Vegetable and spice capsicums. CABI Publishing, Oxon, UK and New York. 204 p.

Cotty, P. J. and Garcia, R. J. 2007. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *Int. Jour. Food Microbiol* 119: 109–115

EC (European Commission) 2006. The Commission Decision, 2006/504/EC Official *Jour. European Union* L199, pp. 21-32.

Fazekas, B., Tar, A. and Kovacs, M. 2005. Aflatoxin and ochratoxin A content of spices in Hungary. *Food Addit. Contam*. 22: 856-863.

FDA (Food and Drug Administration). 2004. Compliance guidance manual. http://www.cfsan.fda.gov.

Hornero-Méndez, D., Gómez-Ladrón de Guevara, R. and Mínguez- Mosquera, M. I. 2000. Carotenoid biosynthesis changes in five red pepper (*Capsicum annuum* L.) cultivars during ripening. Cultivar selection for breeding. *Jour. Agric. Food Chem*. 48: 3857-3864.

Howard, L. R., Smith, R. T., Wagner, A. B., Villalon, B. and Burns, E. E. 1994. Provitamin A and ascorbic acid content of fresh pepper cultivars (*Capsicum annuum*) and processed jalapenos. *Jour. Food Sci*. 59: 362-365.

IARC. 1993. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins, p. 397-444. In IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 56 International Agency for Research on Cancer, Lyon, France.

Koci-Tanackov, S.D., Dimi, G.R. and Karali, D. 2007. Contamination of spices with moulds potential producers of sterigmatocystin. APTEFF. 38: 29–35.

Marin, A., Ferreres, F., Tomas-Barberan, F.A., Gil, M.I. 2004. Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L). *Jour. Agric. Food. Chem*. 52, 3861-3869.

Ozbey, F. and Kabak, B. 2012 Natural co-occurrence of aflatoxins and ochratoxin A in spices. *Food Control* 28: 354–361

Roberts, B.A. and Patterson, D.S.P. 1975. Detection of twelve mycotoxins in mixed animal feed stuff's using a novel membrane cleanup procedure. *Jour. Off. Anal. Chem.* 58: 1178-1181.

Ramagnoli, B., Menna, V., Gruppioni, N. and Bergamini, C. 2007. Aflatoxins in spices, aromatic herbs, herb-teas and medicinal plants marketed in Italy. *Food Control*. 18: 697-701.

Schweiggert, U., Mix, K., Schieber, A., Carle, R. 2006. An innovative process for the production of spices through immediate thermal treatment of the plant material. *Innovative Food Sci. Emerging Technol*. 6: 143-153.

Sinha, A.K. 1995. Mycotoxin- A health hazard. *Yojna*. June issue: 15.

Stack, M.E and Pohland, A.E. 1975. Collaborative study of a method for chemical confirmation of the identity of aflatoxin. *Jour. Assoc. Off. Anal. Chem.* 58: 110-113.

Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R.V., Breiman, R., Brune, M., DeCock, K., Dilley, A., Groopman, J., Hell, K., Henry, S.H., Jeffers, D., Jolly, C., Jolly, P., Kibata, G.N., Lewis, L., Liu, X., Luber, G., McCoy, L., Mensah, P., Miraglia, M., Misore, A., Njapau, H., Ong, C., Onsongo, M.T.K., Page, S.W., Park, D., Patel, M., Phillips, T., Pineiro, M., Pronczuk, J., Schurz Rogers, H., Rubin, C., Sabino, M., Schaafsma, A., Shephard, G., Stroka, J., Wild, C., Williams, J.T., Wilson, D. 2006. Public health strategies for reducing aflatoxin exposure in developing countries. *Environ. Health Perspectives*. 114:1989-1903.

W.H.O. 1979. Environmental health criteria 11. Mycotoxins. World Health Organization, Geneva.

Wu, F. and Khlangwiset P. 2010. Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: Case studies in biocontrol and postharvest interventions. *Food Addit. Contam*. 27: 496-509.