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A study on Aflatoxin Content in Different Oilseeds available in Domestic Market in India

Ashish Mukherjee*, Savita Latkar and Priya Maurya
Central Agmark Laboratory, North Ambazari Road, Nagpur-440010
***Corresponding author: E-Mail: cal@nic.in, Tel: 0712-2561748, Fax: 0712-2982066**

ABSTRACT

Oil seeds are main producer of oils and fats which is essential part of our diet. Presence of aflatoxin in oil seeds may lead to health hazard.

In the present study, the analysis of Aflatoxin B1 content was performed in different oil seeds (Ambadi seed, Cotton seed, Karanj seed, Linseed, Mahua seed, Niger seed, Safflower seed, Sesame seed and Taramira seed) procured from various regions of India and also to assess whether the oilseeds were safe for human consumption. Out of 37 samples analysed in Ambadi seed, 35 samples were showing Aflatoxin below detection level, and 2 samples have aflatoxin of range in between 5.55 ppb to 16.40 ppb. The 72 samples have analysed for Cotton seed, among them 65 shows Aflatoxin below detection level, and 7 samples have aflatoxin of range in between 10.21 ppb to 52.51 ppb. The 17 samples have analysed for Karanj seed, among them 11 sample shows Aflatoxin below detection level, and 6 samples have aflatoxin of range in between 15.24 ppb to 160.28 ppb. The 77 samples have analysed for Linseed, among them 73 samples have Aflatoxin below detection level, and 4 samples have aflatoxin of range in between 10.1 ppb to 47 ppb. The 6 samples have analysed for Mahua seed, among them 4 samples have Aflatoxin below detection level, and 2 samples have aflatoxin of range in between 4.38 ppb to 10.90 ppb. The 14 samples have analysed for Niger seed, among them 9 samples have Aflatoxin below detection level, and 5 samples have aflatoxin of range in between 11.55 ppb to 154.14 ppb. The 38 samples have analysed for Safflower seed, among them 33 samples have Aflatoxin below detection level, and 5 samples have aflatoxin of range between 2.29 ppb to 55.64 ppb. The 84 samples have analysed for Sesame seed, among them 79 samples have Aflatoxin below detection level, and 5 samples have aflatoxin of range in between 1.32 ppb to 107.35 ppb. The 6 samples have analysed for Taramira, all samples have Aflatoxin below detection level. In India, a maximum permissible limit of 30 µg/kg has been indicated under the Food Safety and Standards (Contaminants, Toxins and Residues) Regulation 2011, for all foods meant for human consumption. From our study, it has been revealed that the percentage contaminaton of aflatoxin above the specified limit of FSSAI is more in Karanj seed and Niger seed.

KEY WORDS: Oilseeds, Aflatoxin, HPTLC.

1. INTROUCTION

Oils and Fats are an essential part of our diet, supplying nutrients, improving flavor, aiding in the absorption of vitamins, and providing concentrated sources of energy for our body. Vegetable oil is a triglyceride extracted from a plant. (a) The term "vegetable oil" indicated as plant oils, which are liquid at room temperature, (b) or mostly defined without consider to a substance's state of matter at a given temperature. (c) Oil seeds are of great economic importance and their significance in life of human next to cereals. Oil is extracted mainly from seeds. The vegetable oil is generally use in domestic cooking, and they are also used as a main constituent of soap, body oils and detergents. Oils and fats are natural products and the contamination with impurity will depend with oil type, weather, soil, harvesting, and feed, storage and extraction conditions.

Aflatoxins are toxic and carcinogenic metabolic products of *Aspergillus* (*A. flavus*, *A. parasiticus* and *A. nomius*). The aflatoxins consist of about 20 similar compounds, but usually four are found in foods. These are aflatoxins B1, B2, G1 and G2. Aflatoxin B1 is the most commonly found in food and also the most toxic and classified by the International Agency for Research on Cancer (IARC) as 1st class carcinogen. Aflatoxin producing fungi may contaminate oil seeds under favorable condition, in growing stage, while in storage/ or processed. Aflatoxin contamination results in illness or death in humans and animals and thus is an important public health concern. Prolonged storage and/or contamination during storage or transport have also been associated with higher Aflatoxin levels.

The study of Aflatoxin levels in oil seed was conducted due to the harmful effects of Aflatoxin on human health and wide consumption of oils in Indian market. Frequent monitoring was thus carried out to assess the levels of contamination of Aflatoxin in Vegetable oil, which is directly being consumed by human being, the oil seeds from where it is derived should not be contaminated with Aflatoxin or contain the permissible limit of same. The Food Standard and Safety Authority of India (FSSAI) is responsible for enforcing safety laws and regulations on the production, sale, composition and content of foods and food products as outlined in the Food Safety and Standard Act, 2006; Food and Safety and Standard Regulations, 2011. It also establishes health-based limits for contaminant residues in food. In India, a maximum permissible limit of 30 µg/kg has been fixed under the Food Safety and Standards (Contaminants, Toxins and Residues) Regulation 2011, for all foods meant for human consumption.

The vegetable oils should be safe for human consumption, as such the oil seeds from where the oils have been derived, should not contaminated of Aflatoxin or contain the permissible limit of same. In the present study, Aflatoxin B1 contamination have been carried out in different varieties of oil seeds like Ambadi seed, Cotton seed, Karanj seed, Linseed, Mahua seed, Niger seed, Safflower seed, Sesame seed, and Taramira seed collected from different region of India and also to assess whether the oil seeds were safe for human consumption.

2. MATERIALS AND METHODS

Total 351 Oil seed samples consisting of 37 Ambadi seed, 72 Cotton seed, 17 Karanj seed, 77 Linseed, 6 Mahua seed, 14 Niger seed, 38 Safflower seed, 84 Sesame seed, and 6 Taramira seed were investigated for Aflatoxin B1 levels. The Oil seeds were procured from different regions of country.

Extraction of aflatoxins from Oil seeds: For detection and estimation of aflatoxins from Oil seeds, samples collected from different parts of India, the analytical procedure of solvent extraction and subsequent analysis by HPTLC was employed. About 20 g. dried finely crushed sample accurately weighed in 500 ml. Conical flask containing mixture of 1 gm NaCl, 50 ml Hexane and 125 ml Methanol: Water (55:45) and allowed to stand for 30 minutes with intermittently shaken Thereafter, the mixture was filtered through Whattman filter paper and solution has been taken in separating funnel. Discard Hexane layer. Wash again with Hexane, if require. Collected Methanol: Water layer. 25 ml of this layer taken in separating funnel, and added 25 ml of Chloroform and shake. After layer being separated, discarded the aqueous layer, and Chloroform layer collected. The chloroform layer evaporated to dryness on water bath. The residue was dissolved with 2.5 ml of chloroform and stored in darkness for quantitative analysis.

Quantitative estimation of aflatoxins: The analysis of aflatoxin was being carried out by High performance thin layer chromatography (HPTLC). The analytical equipment for HPTLC (CAMAG Linomat 5) with CAMAG TLC Scanner 3/081123 and operated with win CATs software.

Method of Spotting and Development of TLC plate: Pre-coated TLC sheets silica gel Merck 60 F_{254} 10x10 cm was taken.

Sample application: Apply band with CAMAG Linomat, distance from lower edge of sheet 12 mm, and distance from left edge 12 mm. Spotted 10 µl volume samples extract with band length 5 mm.

Standards application: Apply side by side, 3.0, 6.0 and 10.0 µl standard Aflatoxin B_1 (Concentration 0.5µl/ml).

Chromatography: The development chamber should be filled up with chloroform-acetone (9:1) up to a depth of about 8 mm and insert the sheet, the solvent migrates up to 70 mm. Then plate is air dried.

Scanning of TLC: Mounted air dried plate on Scanner Tray and fixed with the magnets. Scanned plate in TLC scanner, under UV light at 366 nm.

Calculation: The concentration of Aflatoxin B_1 in µg/kg has been calculated as follows:

$$\mu g/kg = \frac{B \times Y \times S \times V}{Z \times X \times W}$$

Where, B = average Area/Height of Aflatoxin B_1 peaks in test aliquots; Y = concentration of Aflatoxin B_1 standards (µg/ml); S = µl of Aflatoxin B_1 standards spotted; V = final volume of test solution (µl); Z = average Area/Height of Aflatoxin peaks in standards aliquots; X = µl test solution spotted; W = gm test portion represented by test solution.

The final results have been obtained by taking average of concentration of Aflatoxin after calculation with respect to Height and Area.

3. RESULTS AND DISCUSSION

The results of Aflatoxin in different varieties of oil seeds have been mentioned in Table.1.

Table.1. Results of Aflatoxin in Oil Seeds

S. No.	Type of Oil seeds	Sample Size	No. of Samples Showing presence of Aflatoxin	Min. value (ppb)	Max. Value (ppb)	% of samples Showing presence of Aflatoxin	% of samples having Aflatoxin > 30 ppb
1	Ambadi	37	2	5.55	16.40	5.40	-
2	Cotton	72	7	10.21	52.51	9.72	2.78
3	Karanj	17	6	15.24	160.28	35.29	17.65
4	Linseed	77	4	10.1	47.0	5.19	1.30
5	Mahua	06	2	4.38	10.90	33.33	-
6	Niger	14	5	11.55	154.14	35.71	21.43
7	Safflower	38	5	2.29	55.64	13.16	2.63
8	Sesame	84	5	1.32	107.35	5.95	1.19
9	Taramira	06	ND	-	-	-	-

ND- Not detected i.e. "0"

The aflaoxin in 9 varieties of oilseeds have been analysed using HPTLC, the results of which i.e. Minimum, Maximum value and % of samples gives positive results of Afltatoxin have been mentioned in Table.1. Out of 37 samples analysed in Ambadi seed, 35 samples were showing Aflatoxin below detection level, and 2 samples have aflatoxin of range in between 5.55 ppb to 16.40 ppb. The 72 samples have analysed for Cotton seed, among them 65 shows Aflatoxin below detection level, and 7 samples have aflatoxin of range in between 10.21 ppb to 52.51 ppb. The 17 samples have analysed for Karanj seed, among them 11 sample shows Aflatoxin below detection level, and 6 samples have aflatoxin of range in between 15.24 ppb to 160.28 ppb. The 77 samples have analysed for Linseed, among them 73 samples have Aflatoxin below detection level, and 4 samples have aflatoxin of range in between 10.1 ppb to 47 ppb. The 6 samples have analysed for Mahua seed, among them 4 samples have Aflatoxin below detection level, and 2 samples have aflatoxin of range in between 4.38 ppb to 10.90 ppb. The 14 samples have analysed for Niger seed, among them 9 samples have Aflatoxin below detection level, and 5 samples have aflatoxin of range in between 11.55 ppb to 154.14 ppb. The 38 samples have analysed for Safflower seed, among them 33 samples have Aflatoxin below detection level, and 5 samples have aflatoxin of range between 2.29 ppb to 55.64 ppb. The 84 samples have analysed for Sesame seed, among them 79 samples have Aflatoxin below detection level, and 5 samples have aflatoxin of range in between 1.32 ppb to 107.35 ppb. The 6 samples have analysed for Taramira, all samples have Aflatoxin below detection level. In India, a tolerance limit of 30 µg/kg has been prescribed under the Food Safety and Standards (Contaminants, Toxins and Residues) Regulation 2011, for all foods meant for human consumption.

4. CONCLUSION

In present study, the nine types of Oil seeds i.e. Ambadi seed, Cotton seed, Karanj seed, Linseed, Mahua seed, Niger seed, Safflower seed, Sesame seed, and Taramira seed are collected from various regions of India, and the content of Aflatoxin has been determined using HPTLC. While going through the results obtained for Aflatoxin in oil seeds, it has been found that except Taramira seeds all other seeds gives positive results for aflatoxin, and among them more than 30% of Karanj, Mahua and Niger seeds are being contaminated with Aflatoxin. It has also been observed from results that 17.65% of Karanj seeds, and 21.43% of Niger seeds have been contaminated with Aflatoxin having value higher than safe limit. About 1-3% of Sesame, Safflower, Linseed and Cotton seeds have been contaminated with Aflatoxin having value higher than safe limit i.e. 30 ppb. It is therefore important for regulatory bodies in India to continuously monitor for aflatoxins in oil seeds available in the consumer market since it is a food safety concern. In addition, post-harvest procedures such as drying techniques and storage should be carefully controlled to minimize fungal growth and thus prevent mycotoxin contamination. The prevention of mycotoxin production at farm level is the best way to control mycotoxin contamination in agricultural products. Also, the other methods may be employed to inactivate aflatoxins, or reduce their levels, in postharvest foodstuffs; particularly while they are in storage.

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REFERENCES

Alfred Thomas, Fats and Fatty Oils, Ullmann's Encyclopedia of Industrial Chemistry, Weinheim: Wiley-VCH, 2002.

Aydin A, Erkan ME, Baskaya R, Ciftcioglu G, Determination of aflatoxin B1 levels in powdered red pepper, Food Control, 18, 2007, 1015-1018.

Baydar T, Engin AB, Girgin G, Aydin S, Sahin G, Aflatoxin and ochratoxin in various types of commonly consumed retail ground samples in Ankara, Turkey, Ann Agric Environ Med., 12 (2), 2005, 193-197.

Baydar T, Erkekoglu P, Sipahi H, Sahin G, Aflatoxin B_1, M1 and ochratoxin A levels in infant formulae and baby foods marketed in Ankara, Turkey, J Food Drug Analy., 15 (1), 2007, 89-92.

FSSI, Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, New Delhi, 2011.

Giray B, Girgin G, Engin AB, Aydin S, Sahin G, Aflatoxin levels in wheat samples consumed in some regions of Turkey, Food Control, 18, 2007, 23-29.

IARC (International Agency for Research on Cancer), Aflatoxins, Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins, IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, 1993, 245-395.

Nuryono N, Agus A, Wedhastri S, Maryudani YB, Sigit Setyabudi FMC, Bohm J, Razzazi-Fazeli E, A limited survey of aflatoxin M1 in milk from Indonesia by ELISA, Food Control, 20, 2009, 721-724.

Ozdemir M, Determination of aflatoxin M1 levels in goat milk consumed in Kilis province, Vet J Ankara Univ., 54, 2007, 99-103.

Parwez Saroj, The Pearson Guide to the B.Sc. (Nursing) Entrance Examination, Pearson Education India, Year, 109.

Robin Dand, The International Cocoa Trade, Wood head Publishing. 1999, 169.

Tajik H, Rohani SMR, Moradi M, Detection of aflatoxin M1 in raw and commercial pasteurized milk in Urmia, Iran, Pakistan J Biol Sci., 10 (22), 2007, 4103-4107.

Yapar K, Elmah M, Kart A, Yaman H, Aflatoxin M_1 levels in different type of cheese products produced in Turkey, Med Wet, 64 (1), 2008, 53-55.