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A STUDY ON AFLATOXIN CONTENT IN WHITE PEPPER AVAILABLE IN DOMESTIC MARKET OF INDIA

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ABSTRACT

White pepper, or sometimes called pepper corn, is a kind of pepper that comes from tropical berries called Piper nigrum. White pepper is one of the world's most favorite spices in history White Pepper is the most exported and traded condiment in the international scene. White peppers are best used for vegetables, sauces and casseroles. White pepper has low sodium content and contains about 0.2 mg of sodium only. It is therefore ideal for those taking a low sodium diet. It also acts as a food preserver. It is also used as an alternative medicine which helps improve flatulence and intestinal upsets. In some cases, it relieves nausea and may even help reduce fever and chills. The aflatoxins are a group of chemically similar toxic fungal metabolites (mycotoxins) produced by certain moulds of the genus Aspergillus growing on a number of raw food commodities. Aflatoxins are highly toxic compounds and can cause both acute and chronic toxicity in humans and many other animals. The aflatoxins consist of about 20 similar compounds belonging to a group called the difuran coumarins, but only four are naturally 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. The White Pepper, which is being used in different types of foods consumed by human being, to be free from Aflatoxin contamination or contain the permissible limit of same. In the present study Aflatoxin B1 contamination have been carried out in two varieties of White Pepper (Whole and Ground) collected from different parts of India and also to assess whether the White Pepper were safe for human consumption.

The aflatoxin in White Peppers have been analyzed using HPTLC. 37 Samples of White Pepper (Whole) analyzed for the estimation of Aflatoxin. 36 samples are having Aflatoxin content below detection level. Only one sample contains 11.223 ppb of Aflatoxin. In FSSAI Max limit for Aflatoxin is 30 ppb.

36 Samples of White Pepper (Ground) analyzed for the estimation of Aflatoxin. Out of 36 samples, 4 samples are having Aflatoxin content. Among them 3 samples have aflatoxin content 78.063 ppb, 40.262 ppb, 81.554 ppb, which are above the limit fixed by FSSAI, and one sample contain aflatoxin 25.112 ppb which is within the limit. 32 samples are having Aflatoxin content below detection level.

Keywords *– White Pepper, Aflatoxin, HPTLC.*

1. INTRODUCTION

Pepper (*Piper nigrum L.*) is the most important spice with economic value and is used as an ingredient in many dishes to give flavor to foods. Piper nigrum L. is also a tropical arbust that forms clusters or racemes in warm climates of 25 to 30°C and 60 to 93% humidity [1,2]. The different types of peppers are due to the different ripening stages of the grains. White Pepper is one kind of Pepper corn that comes from tropical berries called *Piper nigrum*. White pepper consists solely of the seed of the pepper plant, with the darker-colored skin of the pepper fruit removed. This is usually accomplished by a process known as retting, where fully ripe red pepper berries are soaked in water for about a week, during which the flesh of the pepper softens and decomposes. Rubbing then removes what remains of the fruit, and the naked seed is dried. Sometimes alternative processes are used for removing the outer pepper from the seed, including removing the outer layer through mechanical, chemical, or biological methods [3]**.** White pepper consists of two types- Whole and Ground.

Ground white pepper is used in Chinese and Thai cuisine, but also in salads, cream sauces, light-colored sauces, and mashed potatoes (where black pepper would visibly stand out). White pepper has a different flavor from black pepper; it lacks certain compounds present in the outer layer of the drupe. They have a milder flavor than the less ripe version (black peppercorns). White pepper is used as an alternative to black pepper in recipes such as white sauces or white mashed potatoes when a more pleasing appearance is desired to eliminate the black specks throughout the food. White pepper is described as being spicier and fruitier than black pepper, but less complex. White pepper should be added after the dish has been cooked, as overheating can release a bitter flavor.

Aflatoxins are toxic and carcinogenic metabolic products of *Aspergillus (A. flavus, A. parasiticus* and *A. nomius)* [4-6]. Aflatoxins are highly toxic compounds and can cause both acute and chronic toxicity in humans and many other animals. The aflatoxins consist of about 20 similar compounds belonging to a group called the difuranocoumarins, but only four are naturally 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 [7-9].

In spices, fungal growth occurs in warm and humid conditions. Aflatoxin producing fungi may contaminate spices, if grown, stored and/or processed under conditions which favour fungal growth. Hot, humid climates and any pest pressures resulting in bruising or cuts on the commodity will favour the growth of the Aflatoxin producing fungi, either in the field or in storage. Growth of these fungi on certain foods and feeds may result in Aflatoxin production which results in illness or death in humans and animals and thus is an important public health concern [10-12]. Prolonged storage and/or contamination during storage or transport have also been associated with higher Aflatoxin levels.

Spices are products derived from different parts of various plants and are usually contaminated with fungi. Even though spices are generally used in small amounts, they represent an important vector of microbial contamination due to the conditions under which they were grown, harvested and processed [13]. Fungi are the most represented microbial flora isolated from these products. They are able to grow on, and this may subsequently lead to mycotoxin contamination [14]. Since the presence of fungi in spices, i.e., foods containing spices has been associated with formation of noxious odors and other adverse effects including the increased risk for mycotoxin formation under favorable conditions. The predominant contaminants of species are fungi, which are observed as commensal on plants of survived drying and storage. Soil and air are the main inoculums source for causing contamination in crude spices in field. Other practices such, as harvesting, handling and packing cause additional contamination. The spices are collected in tropical areas by simple methods and are commonly exposed to many contaminants before, being dry enough to prevent microbial growth [15]. Fungi are a normal component of food microflora and may be responsible for spoilage and production of mycotoxins [16]**.**

Taking into consideration the potential health risk associated with Aflatoxin, the presence of this mycotoxin in spices has been investigated worldwide. Toma and Abdulla [17] found that Aflatoxin was present in spices sold in Iraqi markets in the Erbil city. Salari et al. [18] also reported the presence of Aflatoxin in spices collected from a farm and sun-dried in Iran. Investigation of Aflatoxin levels in spices was undertaken because of the human health effects of Aflatoxin exposure and the widespread consumption of these products in the Indian market. Frequent monitoring was thus carried out to assess the levels of contaminants in spices in commercial markets of different parts of India. 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 and Drugs Act & Regulations* 2011. It also establishes health-based limits for contaminant residues in food. Tolerances are established as a risk management tool and are generally set only for foods that significantly contribute to the total dietary exposure. The tolerance level for Aflatoxin range from 0 to 50 µg/kg under food safety act. 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 [19].

Concerning the consumption frequency, white pepper is the spice consumed at the highest frequency when compared to the others spices, and to be free from Aflatoxin contamination or contain the permissible limit of same. In the present study, Aflatoxin B1 contamination has been carried out in two varieties of White Pepper (Whole and Ground) collected from different parts of India and also to assess whether the White Peppers were safe for human consumption.

2. MATERIALS AND METHODS

Total 73 nos. White Pepper samples, consisting of 36 nos. White Pepper (Ground) and 37 nos. White Pepper (Whole) were investigated for Aflatoxin B_1 levels. The samples were collected from different parts of country.

2.1. Extraction of aflatoxins from Dates

For detection and estimation of aflatoxins in White Pepper, 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.

2.2. Quantitative estimation of aflatoxins

Quantitative estimation of aflatoxin was done by High performance thin layer chromatography (HPTLC). The analytical equipment for HPTLC (CAMAG Linomat 5) with CAMAG TLC Scanner 171005, CAMAG Visualizer 171113 and operated with winCATs software.

2.3. Method of Spotting and Development of TLC plate

Pre-coated TLC sheets silica gel Merck 60 F_{254} 10x10 cm was taken.

2.3.1. 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.

2.3.2. Standards application

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

2.3.3. 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.

2.3.4. 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 region wise have been mentioned in Tabe-1 for White Pepper- Whole and Ground.

ND- Not detected and may be taken as "0".

36 samples of White Pepper (Ground) and 37 samples of White Pepper (Whole) were collected from the different parts of India. These samples were analyzed for Aflatoxin content. The results of analysis are summarized in Table-1. Out of the 36 White Pepper (Ground) samples analyzed, 4 nos. of samples give positive results for Aflatoxin B1 having value 78.063 ppb, 40.262 ppb, 81.554 ppb and 25.112 ppb. In case of White Pepper (Whole), only one sample gives positive results out of 37 samples analyzed, having value 11.223 ppb. The Aflatoxin contamination is due to ubiquitous prevalence of toxigenic *Aspergillus flavus* as a natural contaminant in the samples. Although only one sample of White Pepper (Whole) is contaminated, but the level is within the limits of 30 µg/kg set by FSSAI. But in White Pepper (Whole), the three samples out of four, which tested positive for Aflatoxin, give much higher value than the limit set by FSSAI. Limited reports have been published about the occurrence of aflatoxins in White Pepper. It has been revealed from the recent study **(20)** that White pepper is more susceptible for Aflatoxin contamination than Black Peeper. It has been observed from present study that Aflatoxin contamination is more marked in ground samples than in whole ones because the moulds were more exposed to these molecules.

Table-1: Aflatoxin content in White Pepper (Whole and Ground)

Aflatoxin in White Pepper (Ground)				Aflatoxin in White Pepper (Whole)		
Sl. No.	**Region**	**Result (in ppb)**		**Sl. No.**	**Region**	**Result (in ppb)**
1	Amritsar	78.063		1	Amritsar	ND
2	Amritsar	ND		2	Amritsar	ND
3	Amritsar	ND		3	Amritsar	ND
4	Amritsar	ND		4	Amritsar	ND
5	Amritsar	ND		5	Bhopal	ND
6	Bhopal	ND		6	Bhopal	ND
7	Bhopal	ND		7	Guntur	ND
8	Guntur	ND		8	Guntur	ND
9	Guntur	ND		9	Guntur	ND
10	Guntur	ND		10	Guntur	ND
11	Guntur	ND		11	Guntur	ND
12	Guntur	ND		12	Kanpur	ND
13	Kanpur	40.262		13	Kanpur	ND
14	Kanpur	ND		14	Kanpur	ND
15	Kanpur	ND		15	Kanpur	ND
16	Kanpur	ND		16	Kanpur	ND
17	Kanpur	ND		17	Kanpur	ND
18	Kanpur	ND		18	Kanpur	ND
19	Kanpur	ND		19	Kanpur	ND
20	Kanpur	ND		20	Kochi	ND
21	Kochi	81.554		21	Kolkata	ND
22	Kolkata	ND		22	Kolkata	ND
23	Kolkata	ND		23	Kolkata	ND
24	Kolkata	ND		24	Mumbai	ND
25	Mumbai	ND		25	Mumbai	ND
26	Mumbai	ND		26	Mumbai	ND
27	Mumbai	ND		27	Mumbai	ND
28	Mumbai	ND		28	Mumbai	ND
29	Mumbai	ND		29	Mumbai	ND
30	Mumbai	ND		30	Mumbai	ND
31	Mumbai	ND		31	Mumbai	ND
32	Nagpur	25.112		32	Mumbai	ND
33	Nagpur	ND		33	Nagpur	11.223
34	Nagpur	ND		34	Nagpur	ND
35	Nagpur	ND		35	Nagpur	ND
36	Nagpur	ND		36	Nagpur	ND
37				37	Nagpur	ND

4. CONCLUSION

In present study, the two varieties of White Pepper i.e. whole and ground are collected from different parts of India mentioned in Table-1, and the content of Aflatoxin has been determined using HPTLC. 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. White pepper is the spice consumed more frequently when compared to the others spices, and to be free from Aflatoxin contamination or contain the permissible limit fixed by FSSAI. While going through the results obtained for Aflatoxin in White Pepper – Whole and Ground, it has been found that only one sample of White Pepper (Whole) gives positive results, which has been collected from "Nagpur" region, but the value is within the permissible limit fixed by FSSAI. But in case of White Peeper (Ground), 4 samples give positive results of Aflatoxin, collected from Amritsar, Kanpur, Kochi and Nagpur. Out of these 4 samples, three samples give the value of Aflatoxin, which are much higher than the permissible limit fixed by FSSAI. This can be explained

by the fact that ground samples had the highest nutrient availability; as such it is more susceptible for fungal growth and mycotoxin contamination. About 10% of ground white pepper has been contaminated with Aflatoxin having value much higher than safe limit. It should be controlled for safety of health of people of India. It is therefore important for regulatory bodies in India to continuously monitor for aflatoxins in spices 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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