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Detection of Aflatoxin in Paneer and Other Milk Products Marketed In Madurai North, India

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ABSTRACT
Aflatoxins are cancerous secondary metabolites produced by Aspergillus sp. Peanuts, corn, and cotton seeds can be contaminated with aflatoxins before harvest, and during the storage period. When lactating animals consume contaminated feed, the resulting milk will contain aflatoxin. Milk products prepared using contaminated milk also contain aflatoxin. Minimal sources are available regarding aflatoxins in paneer, one of India's main culinary products. This research aims to detect aflatoxin in milk products and especially in paneer. In India, there is a practice of preparing paneer from milk. Hence different milk brands were used to prepare paneer to check for its presence. In 2020, popular brands of milk products including milk, paneer, butter, and cheese available in supermarkets of Madurai North were collected. Extraction of aflatoxin via organic solvents and Thin Layer Chromatography was done. Fluorescence and confirmatory tests were done to confirm their presence. Out of 25 samples tested, 15 (60%) showed positive results for aflatoxins. This indicates the high prevalence of aflatoxin in paneer and other milk products and emphasizes the need for quantification studies and continuous monitoring of aflatoxins in milk, paneer, and other milk products available in Madurai North, India.

Keywords: *Aflatoxin, Paneer, Milk products, Madurai, Cancer*

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I. INTRODUCTION

Aflatoxins are cancerous secondary metabolites produced predominantly by two fungal species: Aspergillus *flavus* and *Aspergillus parasiticus*(Alshannaq*et al*., 2017). Most common aflatoxins include aflatoxin B1, B2, G1, G2, M1 and M2 (metabolites of B1 and B2) (Rajarajan*et al*., 2013). In nature, aflatoxin B1 is the most widespread in the world which accounts for 75% of all aflatoxin contamination of food and feeds. Aflatoxin B1 and B2 fluoresce blue under UV light while aflatoxin G1 and G2 fluoresce green under UV light. (Wacoo*et al*., 2014). Aflatoxin M1 is strongly fluorescent, emitting blue violet under UV light (Marchese *et al.*, 2018).

Aflatoxin B1 reacts with DNA (methylation) resulting in G-T transversion. Point mutation of G to T transversion results in aflatoxin-induced hepatocellular carcinoma. Aflatoxin B1 promotes AGG-AGT transversion (arginine-serine). This point mutation occurs in the P^{53} gene at the 249^{th}codon. The P^{53} gene is responsible for DNA repair. Apart from G-T transversion, G-C transversion, and G-A transition have also been reported (Levy *et al.*, 1992).

Due to aflatoxins' carcinogenicity, the IARC (International Association for Research on Cancer) of WHO (World Health Organisation), classified aflatoxins as a Group 1 carcinogen which implies that it can cause cancer in humans (Ostry*et al*., 2017).

When the lactating animal feeds on contaminated foodstuffs which contain aflatoxin B1, biotransformation of aflatoxin B1 takes place in the liver cells which converts aflatoxin B1 into aflatoxin M1 and finally, aflatoxin M1 gets excreted *via* milk (Dhanasekaran*et al.*, 2011).The aflatoxin M1 contaminated milk affects both young ones and consumers who receive the contaminated milk. Aflatoxin M1 and M2 are stable in milk processing techniques which indicates that even pasteurized milk is not aflatoxin-safe (Galvano *et al*., 1996).

Both aflatoxin B1 and M1 can be present in the milk if the precursor (aflatoxin B1) is abundant enough to reach beyond the metabolic capacity of the animal (Scaglioni *et al*., 2014). These aflatoxins enter milk products like cheese, and paneer when milk products are prepared from contaminated milk (Applebaum *et al*., 1982).

Paneer represents the South Asian variety of cheese widely used in several culinary preparations and snacks (Khan *et al*., 2011). Since there is the practice of preparing paneer from milk is common in India, milk

samples (pasteurized & unpasteurized) available in the study area were collectedto prepare paneer. These samples were also included in the study.

High temperature and humidity levels in tropical countries favour the growth of aflatoxin-producing fungal species (Espinosa-calderon*et al*., 2011). Thus,the detection of aflatoxins in food products is important especially when it comes to tropical countries like India.

Since very limited sources are available regarding aflatoxin in paneer which is the type of cheese indigenous to the Indian subcontinent, detecting aflatoxins in paneer could form the basis for future research projects. Hence this study aims to detect aflatoxins in paneer and other dairy products in Madurai North, India where this kind of studyhas not been done before.

II. METHODOLOGY

SAMPLING

In the year 2020 (January-May), popular milk, butter, paneer, and cheese brands were collected from supermarkets in Madurai North, India. This also included unpasteurized milk samples available in local shops. In this study, 5 (pasteurized) branded milk samples, 5 (unpasteurized) unbranded milk samples, 5 branded butter samples, 5 branded cheese samples, and 5 branded paneer samples were included (n=25). Collected milk samples were used to prepare the paneer. Prepared paneer samples and other milk product samples were analyzed for the presence of aflatoxin.

PREPARATION OF PANEER

The milk sample was allowed to boil.A few lemon drops were added to reduce the pH(acidification leads to the curdling of milk). The milk sample was stirred continuously till the whey water was wholly separated. Whey water was completely drained using a muslin cloth.This paneer sample was used for the extraction of aflatoxins.

TLC PLATE PREPARATION

Microscopic glass slides were used for TLC(Thin Layer Chromatography) plate preparation.Glass slides were soaked in chromic acid overnight to remove the contaminants. Before usage, glass slides were thoroughly washed and allowed to dry. Silica gel slurry was prepared by mixing silica gel G (6 grams) and sterile distilled water (12 ml) vigorously for no longer than a minute. TLC plates were prepared by using the pouring technique (in which slurry was poured on a plate and allowed to flow over it so that the slurry was evenly covered). Prepared plates were allowed to air dry overnight. Plates were activated at 100°C for 1 hour before using for TLC analysis.

EXTRACTION OF AFLATOXINS

Shih and Marth (1971) have proposed a procedure for the rapid recovery of aflatoxins from cheese and other foods and that extraction method was followed in this study.

SPOTTING AND PLATE DEVELOPMENT

A slight indentation was made on the plate as a line of origin.Using a micropipette, sample extract was spotted on the TLC plate (each sample was allowed to run in triplicates).The developing solvent was prepared using chloroform: methanol: water (98:1:1).Developing solvent was poured into the beaker, then closed and allowed to stand for nearly 10 minutes to attain saturation. Spotted TLC plates were kept inside and allowed to run until the mobile phase reached 5-7mm from the top of the TLC plate.Later, developed TLC plates were taken out and allowed to evaporate the solvent. Developed plates were subjected to undergo fluorescence test.

FLUORESCENCE TEST

Developed plates were checked for aflatoxins by placing them under UV light. Aflatoxins fluoresce blue or blue green under UV light. Plates that showed positive results for aflatoxins were subjected to undergo a confirmatory test.

CONFIRMATORY TEST (Sinha, 1999)

Plates were sprayed with 20% sulphuric acid. Sprayed plates were heated at 100°C for 10 minutes. Then, plates were placed under UV light to detect aflatoxins. Aflatoxins fluoresce yellow under UV light after performing a confirmatory test.

III. RESULTS AND DISCUSSION

Out of 25 samples tested, 15 samples (60%) showed positive results for aflatoxin (Figure 1 and Table 1). 2 out of 5 samples (40%) of branded paneer, 3 out of 5 samples (60%) of paneer from pasteurized milk, 5 out of 5 samples (100%) of paneer from unpasteurized milk, 3 out of 5 samples (60%) of branded butter, 2 out of 5samples (40%) of branded cheese showed positive results for aflatoxin (Figure 2, Figure 3, Figure 4, Figure 5, and Figure 6depicts positive and negative results for aflatoxin under fluorescence and confirmatory tests).This indicates that milk samples from which milk products are prepared could contain aflatoxin. If milk samples contain aflatoxin, this means that the cow from which milk was obtained could consume contaminated feedstuffs that contain aflatoxin. This ultimately stipulates that there is a need for the regulation of aflatoxin levels in food and fodder before reaching livestock. A similar study was conducted by Peter *et al*. (2015) in which they found aflatoxin (B1, B2, G1, G2) in 43% of marketed paneer samples in Chennai.

100% of paneer samples from unpasteurized milk and 60% of paneer samples from pasteurized milk showed positive results for aflatoxin. Similar results were obtained by Kafle*et al*. (2012) in which they reported the presence of aflatoxin in both raw (50%) and pasteurized (35.75%) milk samples.The presence of aflatoxin in pasteurized milk samples emphasizes that aflatoxins might have thermostable properties (Galvano *et al*., 1996).From the results obtained, it's understood that the prevalence of aflatoxin was high in paneer and other milk products accessible in Madurai North, India, and the presence of aflatoxin in food samples poses a serious health risk among the public.

IV. CONCLUSION

Aflatoxin-related health issues are a global concern. Since tropical climate and increasing globalwarming favours the growth of aflatoxin-producing fungal species, there is an urgent need to ensure proper monitoring facilities to regulate aflatoxin level in food and feedstuffs before reaching consumers and livestock to reduce the exposure of aflatoxins to humans and animals.Mold-resistant crops and proper storage conditions after harvest can reduce the growth of aflatoxin-producing fungal species.Moreover, consumers should be aware of aflatoxins and try to avoid moldy food products as far as possible. The presence of aflatoxin in food samples shows the high chance of getting aflatoxin-related health problems among people in the area from which samples were collected for this study. Further quantitative analysis of milk products in the same area can give quantitative data about aflatoxin contamination, thereby predicting the harmful health effects of aflatoxins.

V. RECOMMENDATIONS

Aflatoxins have serious effects on humans and animals. This study reflects the prevalence of aflatoxin in products marketed in Madurai North, India. Further research studies can help toknow the concentration of aflatoxin in food samples in the same study area. Based on that stringent regulations should be undertaken to avoid aflatoxin-related health risks in near future.

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Table1. Prevalence of aflatoxin in paneer and other milk products

S.No	Sample type	Fluorescence Test	Confirmatory test	Inference
1.	Branded paneer 1	- - -	Nil	Absence of aflatoxin.
2.	Branded paneer 2	+ + +	+ + +	Presence of aflatoxin.
3.	Branded paneer 3	+ + -	--	Absence of aflatoxin.
4.	Branded paneer 4	- - -	Nil	Absence of aflatoxin.
5.	Branded paneer 5	+ + +	+ + +	Presence of aflatoxin.
6.	Paneer from pasteurized milk 1	- - -	Nil	Absence of aflatoxin.
7.	Paneer from pasteurized milk 2	+ + +	- - -	Absence of aflatoxin.
8.	Paneer from pasteurized milk 3	+ + +	+ + -	Presence of aflatoxin.
9.	Paneer from pasteurized milk 4	+ + +	+ + +	Presence of aflatoxin.
10.	Paneer from pasteurized milk 5	+ + +	+ + +	Presence of aflatoxin.
11.	Paneer from raw milk 1	+ + +	+ + -	Presence of aflatoxin.
12.	Paneer from raw milk 2	+ + +	+ + +	Presence of aflatoxin.
13.	Paneer from raw milk 3	+ + +	+ + +	Presence of aflatoxin.
14.	Paneer from raw milk 4	+ + -	+ + -	Presence of aflatoxin.
15.	Paneer from raw milk 5	+ + +	+ + +	Presence of aflatoxin.
16.	Branded butter 1	- - -	Nil	Absence of aflatoxin.
17.	Branded butter 2	+ + +	+ + +	Presence of aflatoxin.
18.	Branded butter 3	+ + +	+ + +	Presence of aflatoxin.
19.	Branded butter 4	+ + +	+ + +	Presence of aflatoxin.
20.	Branded butter 5	+ + -	- - -	Absence of aflatoxin.
21.	Branded cheese 1	+ + +	- - -	Absence of aflatoxin.
22.	Branded cheese 2	+ + +	+ + +	Presence of aflatoxin.
23.	Branded cheese 3	+ + +	+ + +	Presence of aflatoxin
24.	Branded cheese 4	- - -	Nil	Absence of aflatoxin
25.	Branded cheese 5	- - -	Nil	Absence of aflatoxin

Figure 1. Prevalence of aflatoxin in milk products

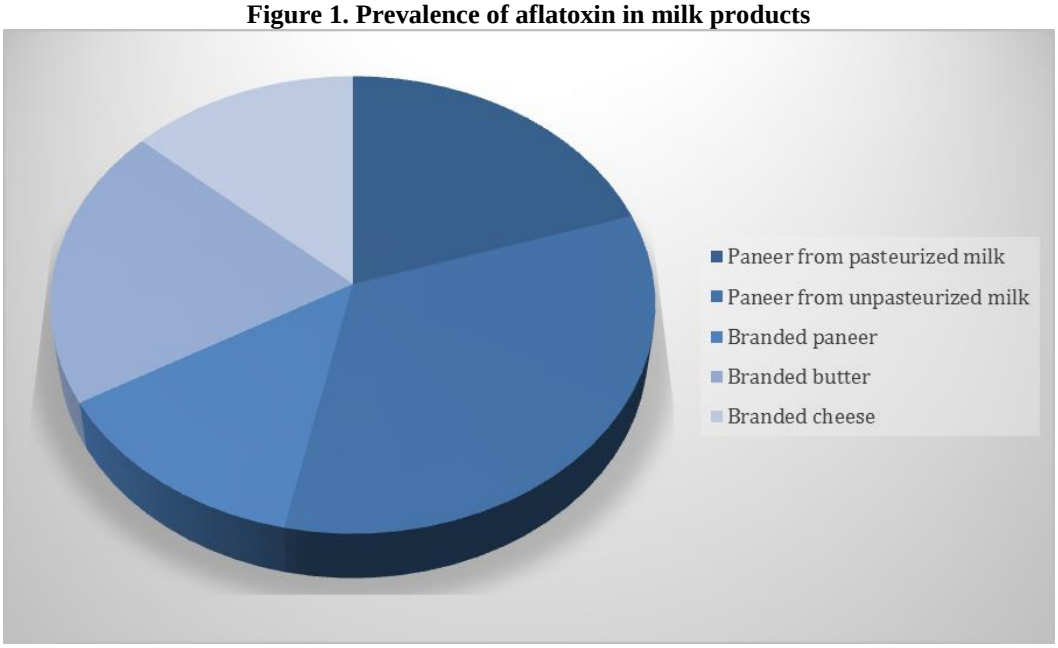

Figure 2. Blue fluorescence under UV light

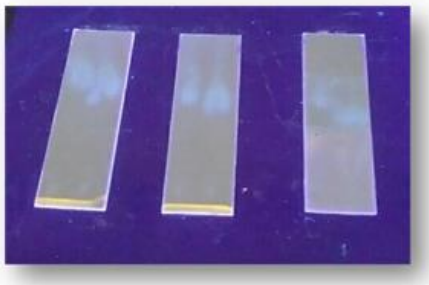

Figure 3. Blue and blue-green fluorescence under UV light

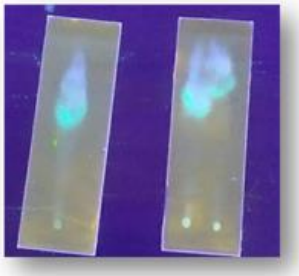

Figure 4. Yellow fluorescence under UV light in the confirmatory test