iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 12 **Issue:** III **Month of publication:** March 2024

DOI: https://doi.org/10.22214/ijraset.2024.59207

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 12 Issue III Mar 2024- Available at www.ijraset.com

A Study on Aflatoxin Content in Urad Dal Flour in India

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Abstract: ***Black gram is commonly known as black lentils, mungo beans, and matpe beans and is scientifically called the Vigna Mungo. Black gram has its origin in India, being the world's largest producer as well as consumerand locally called as Urad. It is one of the most vital varieties of pulse in the Indian subcontinent, and has a lot of nutrients in Urad Dal powder. Mycotoxins are of various types, among with aflatoxin is more popular due to their stability varying ambient temperatures and pH conditions, aflatoxins can build up in food at any point along the supply chain. Aflatoxins can cause liver necrosis, bile duct proliferation, edema or lethargy. There are no reports regarding the level of Aflatoxin content in Urad Dal Flour. The objectives of this study was to determine the concentrations of Aflatoxin B_1 in Urad Dal Flour collected from various parts of India and also to assess whether the urad Flour were safe for human consumption. Out of 21 samples of Urad Dal Flour analysed for Aflatoxin, all samples were found to be free from Aflatoxin i.e aflatoxin content was Below detection limit. As per FSSAI, the maximum permissible limit for Aflatoxin is 30 ppb. The study showed that the Urad Dal Flour samples collected from various regions of India were found to contain aflatoxin within the permissible limit and are safe for human consumption.***
Keywords: Urad Dal Flour, Aflatoxin, HPTLC

I. INTRODUCTION

India is a major legume producing country in the world. Legumes serve as an economical source of complimentary proteins and also add variety to diet for human population. They contribute as high protein constituent in India [1]. Pulses/legumes are the pod bearing plants which are cultivated widely belongs family of leguminosae. Seeds of whole legume plant are of major concern to human as they are a rich source of protein ranging from 20-50% along with amino acids, soluble dietary fiber and certain micronutrients like iron and zinc. They also have low glycemic index. Since they are from plant source, are devoid of cholesterol reducing the risk of cardiovascular disease and diabetes. It is consumed in Indian diet and Western diet since it is a very rich source of protein and performs an essential role in the diet of human nutrition [2].

Black gram (*Vigna mungo* L.) has its origin in India, being the world's largest producer as well as consumerand locally called as Urad. The production ranges about 1.5 to 1.9 million tons of black gram (urad) annually from about 3.5 million hectares of area, with an average productivity of 500 kg per hectare. Black gram output accounts for about 10% of India's total pulse production. In India Maharashtra, Uttar Pradesh, Andhra Pradesh, Orissa, Tamil Nadu, Rajasthan, Chhattisgarh and Madhya Pradesh are the top cultivators and producer of black gram. It is part of diet for millions of people and a cheap source of protein with 17-34% of protein in Seeds. Black gram is perfect combination of all nutrients, which includes proteins (23%), carbohydrates (51%), fat (1.7%), ash (3.17%), zinc (3.00mg), iron (5.97mg) and calcium (55.64mg) [3]. Proteins of black gram are digested in an easy manner and consist of satisfactory amount of sulfur containing amino acids. They are rich in vitamins and phosphoric acid. It is generally used as whole, split beans and dehusked split beans (dal)[2]. Urad dal is milled to obtain urad dal flour and incorporated to various food products for value addition. Papads, thin wafer like products prepared from black gram or combination of black gram with other pulses with and without spices are made in small scale industries or at homes [4]. Flour of black gram was added to biscuits to enhance its nutritional quality [5].

Mycotoxins are of various types, among with aflatoxin is more popular due to their stability varying ambient temperatures and pH conditions, aflatoxins can build up in food at any point along the supply chain. Aflatoxin B1, B2, G1, and G2 are well-known aflatoxin examples. [6]. The most harmful type of aflatoxin causing cancer is produced by the Aspergillus flavus is aflatoxin B1 (AFB1), Aspergillus parasiticus and the rare Aspaergillus nomius [7]. According to the International Agency for Research on Cancer (IARC) classification, AFB1 is a known carcinogenic chemical which involves the mechanism of AFB1 toxicity in the human body causing DNA adducts, which are precursors to cancer cell development, can cause liver damage, growth abnormalities [8], and fertility problems [9].

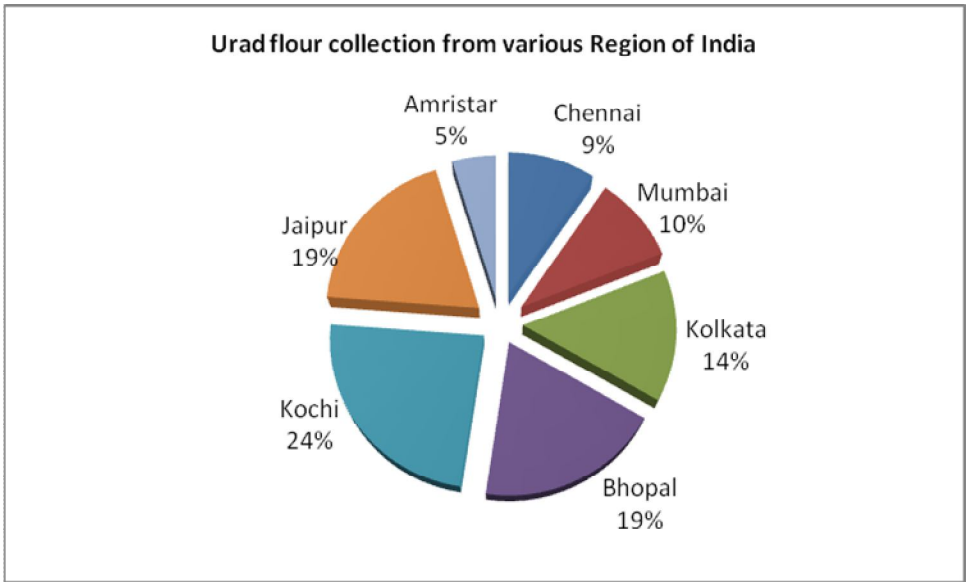
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ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 12 Issue III Mar 2024- Available at www.ijraset.com

During the last decade, the number of toxins has increased, which affects the safety of raw materials and food safety for consumers, so the food safety aspect is a factor considered by consumers when choosing a food product [10]. Contamination occurs during the various stage of processing including milling, packaging, storage, transportation, handling etc..

The bibliography regarding the contamination of Aflatoxin B1 in cereals including paddy as well as rice, wheat, maize and other food materials like maize, peanut are traceable and in urad dal flour is scarce and hence with this objective, a study was carried out for the level of contamination of Aflatoxin B1 in the Urad Dal flour available in India.

II. MATERIALS AND METHODS



A. Extraction of aflatoxins from Urad Dal Flour

For detection and estimation of aflatoxins in 21 no. Urad Dal flour, samples were collected from different parts of India. The solvent extraction of the urad dal flours were done followed by subsequent analysis by HPTLC. 20 g of accurately weighed, homogenized urad dal flours were taken individually in 500 ml Conical flask containing mixture of 1 g NaCl,50 ml Hexane and 125 ml Methanol: Water (55:45). The sample was mixed well and allowed to stand for 30 minutes with intermittently shaking of the mixture. Thereafter, the mixture was filtered and solution was transferred to separating funnel. The layers were allowed to separate. Hexane layer was discarded. Methanol: Water layer was collected seperately. 25 ml of this layer was transferred in another separating funnel and added with 25 ml of Chloroform. It was mixed well and allowed to seperate. The aqueous layer was discarded and Chloroform layer was collected. The chloroform was evaporated to dryness on water bath leaving residue. The residue was dissolved with 2.5 ml of chloroform and stored in darkness for quantitative analysis.

B. Quantitative estimation of aflatoxins

Quantitative estimation of aflatoxin was done by High performance thin layer chromatography (HPTLC-CAMAG Linomat 5) with CAMAG TLC Scanner-3/081123 and operated with winCATs software.

C. Method of Spotting and Scanning of TLC Plate

Pre-coated TLC sheets silica gel Merck 60 F_{254} 10x10 cm were used in the detection of aflatoxin contamination in urad dal flour. Band with CAMAG Linomat was applied with a distance from lower edge and left edge of sheet 12 mm each. Spotted 10 µl volume samples extract with band length 5 mm. Standard Aflatoxin B_1 was applied side by side, 3.0, 6.0 and 10.0 µl. The development chamber was filled with chloroform-acetone (9:1) upto a depth of about 8 mm and the spotted sheet was inseerted. The solvent migrates up to 70 mm. Then plate is air dried. The air dried plate is placed on the Scanner Tray of HPTLC and fixed with the magnets and Scanned in TLC scanner under UV light at 366 nm.

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D. Calculation

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

$$\text{Aflatoxin } B_1(\mu g/kg) = \frac{B \times Y \times S \times V}{Z \times X \times W}$$

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 = g 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.

III. RESULTS AND DISCUSSION:

A total of 21 samples were collected from different parts of India with a distribution of 5% from Amristar,9 % from Chennai, 10 % from Mumbai, 14% from Kolkata, 19% from Bhopal, 24 % from Bhopal, 24 % from Kochi and 19% from Jaipur. Table 1 showed the level of Aflatoxin content in 21 samples of which all samples are found to be below detection limit.

Table 1. Level of Aflatoxin content in ppb in Urad Dal flour samples obtained from different parts of India

S No	Region	Sample Code	Aflatoxin in ppb
1	Mumbai	CALT-213	BDL
2	Bhopal	CALT-248	BDL
3	Bhopal	CALT-249	BDL
4	Bhopal	CALT-104	BDL
5	Kochi	CALT-13	BDL
6	Kochi	CALT-14	BDL
7	Kochi	CALT-15	BDL
8	Kochi	CALT-16	BDL
9	Mumbai	CALT-60	BDL
10	Chennai	CALT-73	BDL
11	Chennai	CALT-74	BDL
12	Jaipur	CALT-83	BDL
13	Jaipur	CALT-84	BDL
14	Jaipur	CALT-85	BDL
15	Jaipur	CALT-86	BDL
16	Jaipur	CALT-87	BDL
17	Amristar	CALT-118	BDL
18	Amristar	CALT-119	BDL
19	Amristar	CALT-120	BDL
20	Amristar	CALT-121	BDL
21	Guntur	CALT-136	BDL

BDL :- Below Detection Limit

The present study indicated that the Aflatoxin B_1 is below the detection limit for all the 21 urad flour samples. The food safety and standard Authority of India had established health based limits for contaminant residues through Food safety and standards Act, 2006 and Food Safety and standards Regulations 2011 as tolerance limit of 30µg/Kg for aflatoxin for all foods meant for human consumption.

The EU established maximum levels of AFB_1 and total AFs (2 and 4 µg/kg, respectively) in cereals for human consumption[11]. Similarly codex has set standards for maize, sorghum, husked and polished rice, There are no specific standards and literatures available for legumes especially for for black gram (urad dal) and urad flour.

However, the literature studies are more common for the contamination of Aflatoxin B_1 in rice indicating, in China, with an average of 0.06 µg/kg , AFB in 235 of 370 samples and an average contamination level of around 0.5-0.6 µg/kg detected in all 29 samples. The presence of aflatoxins has been reported in rice from 12 states in India was about 38.5 % out of 1511 sam-ples were contaminated by AFB. Another survey covered 20 states of India, reported thatAFB was present in 814 of 1200 samples ranging from 0.1 to 308 µg/kg. In Indonesia, AFBwas detected in 2 of 2 rice samples with a range between 2.0–7 0.0 µg/kg. in Iran, AFB in 27 of 30 samples with an average level of 2.9 µg/kg, in South Korea, AFB was present in 5 of 88 samples at the range of 1.8–7.3 µg/kg[12].

IV. CONCLUSION

In the present study for contamination of aflatoxin in uradflour with HPTLC, 21 samples collected from various region of India are having aflatoxin below detection limit ie it is free from contamination and safe for human consumption. The urad flour samples satisfies the FSSAI standards.

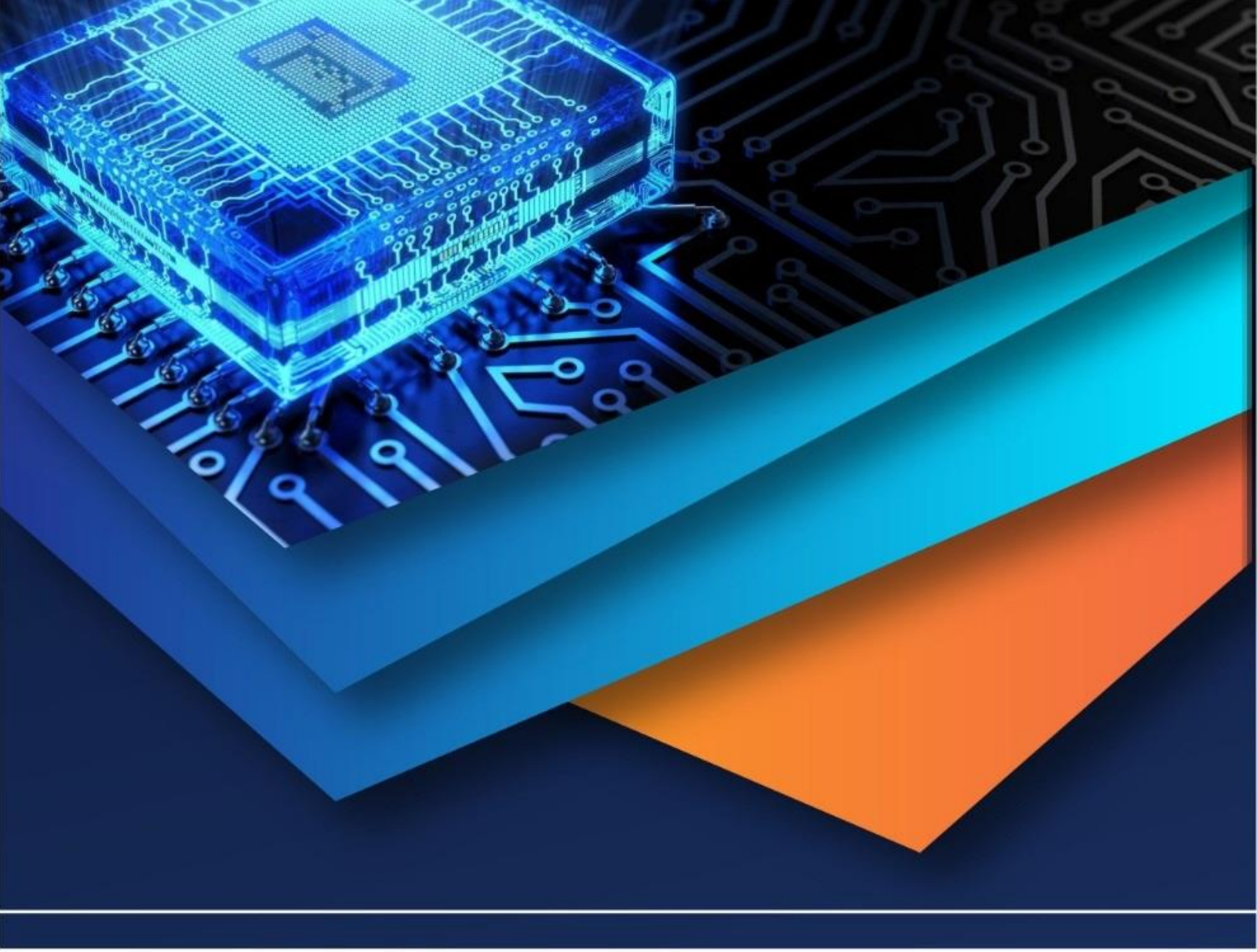
V. ACKNOWLEDGMENTS

We would like to express our sincere gratitude to Shri Faiz Ahmed Kidwai, Additional Secretary - Agricultural Marketing Adviser to the Govt. of India and all the Staffs of Central Agmark Laboratory and Regional Agmark Laboratories who have been source of constant inspiration to us. We would like to express our gratitude to Regional Office, Kolkata, Chennai, Bhopal, Jaipur, Guntur, Amritsur,Mumbai and Kochi to collect and provide the samples of Urad Dal flour for analysis. The views expressed in the manuscript are that of authors and not binding on the Government of India.

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