



# A Study On Analysis Of Aflatoxin B1 In Ragi Flour In India

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**Abstract:** Ragi (Finger millet) is a nutrient rich millet and one of the staples in India. Postharvest handling of the grains may lead to contamination with various mycotoxins especially Aflatoxin B1. Aflatoxin B1 (AFB1) is the most common carcinogen of Aflatoxins, produced by *Aspergillus flavus* and *Aspergillus parasiticus* of fungi, which contaminates many agricultural products in the daily diet of humans. A study was conducted with 34 ragi flour samples collected from various region of India. A maximum of 29% of the collected samples showed a contamination of AflatoxinB1 between 3.01 to 4.00 ppb by HPTLC. The analysed samples were within the limits of AflatoxinB1 contamination standards laid by FSSAI.

**Index Terms** - Aflatoxin B1, HPTLC, Method validation, Ragi, Finger Millet

## I. INTRODUCTION

The global food security has narrowed down due to various factors including availability of staples and others crops and nutritional insecurity imposed by various agronomic, socioeconomic and other factors. Millets provide food and nutritional security[1].

Finger millet (*Eleusine coracana* L.) is commonly known as Ragi in India and one of the staples millet. Ragi has shown significant attention globally for its health benefits. The major component of Ragi is starch, constituting about 70% followed by protein, lipids, vitamins, minerals and other substances in the quality of finger millet-based foods[2].

**Aflatoxins** are probably the most common and widely known mycotoxins contaminant. Aflatoxins are a class of toxic metabolites generated by certain fungi that are commonly found on agricultural crops such as maize (corn), peanut, cotton seed, copra, rice, maize, wheat, sorghum, pulses, figs and oilseed cakes, tree nut [3]. *Aspergillus flavus* and *Aspergillus parasiticus* are the dominant fungal species that produce aflatoxins under drought, warm and humid conditions. Aflatoxin producing fungi can contaminate crops in the field at harvest and during storage [4]. Among various types of Aflatoxins, the predominant ones - Aflatoxin B1, B2, G1 and G2; these four occur in foods and Aflatoxin M1 and M2 two as metabolites in the milk of animals who have been fed contaminated feed. A hydroxylated metabolite of Aflatoxin B1- can pose a severe threat to human and animal health via dietary exposure [5]. The studies in Aflatoxin content in Ragi flour are limited.

With this objective, A study was conducted for the analysis of Aflatoxin B1 in ragi flour in India.

## II. MATERIALS AND METHODS:

The analytical methods for Aflatoxins analysis include thin layer chromatography (TLC), High performance liquid chromatography (HPLC); and High performance Thin liquid chromatography (HPTLC) [6]. HPTLC method is adopted in the current study with reference to AOAC [7].

The reagents include acetonitrile, methanol, toluene, chloroform, acetone, petroleum ether, sodium chloride, sodium sulphate. The standard Aflatoxin b1 procured from sigma aldrich. The apparatus include HPTLC, Analytical balance, 20x10 Twin trough Chamber, Whatmann filter paper No. 4.

## 2.1 EXTRACTION OF AFLATOXIN B1

The collected Ragi flour samples were individually analysed at Central Agmark Laboratory, Nagpur for the presence of aflatoxin B1 with the following procedure. 25 g of the ragi flour was transferred to a 250 ml conical flask. 2 g of sodium chloride (NaCl) and 100 ml of Methanol: Water (80:20) ratio was added to that flask. The mixture was shaken for 10 minutes in orbiter shaker and then sonicated for 10 minutes at 30° C. The mixture is filtered through whatmann filter paper no. 4. 50 ml of the aqueous methanol layer was taken in 100ml volumetric flask and 50 ml PBS buffer (pH 7.2 - 7.4) was added and made up the volume to 100 ml. The 20 ml of the filtered solution is passed through a 0.2 µ syringe filter to remove residual turbidity & pass it through the Aflaclean column.

## 2.2 PREPARATION OF STANDARD AFLATOXIN B1 SOLUTION

The Stock Aflatoxin B1 100ppm solution was prepared by dilution of 1mg to 10ml of Toluene–Acetonitrile (98:02) standard volumetric flask. The Working standard solution for analysis used was 100 ppb.

**2.3 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-3/081123 and operated with winCATs software. The TLC sheets precoated with silica gel Merck 60 F<sub>254</sub> 10x10 cm were used in this study. The software was operated as per user manual and applied band with CAMAG Linomat, distance from lower edge of sheet 12 mm and distance from left edge 12 mm. Spotted 10 µl volume ragi flour samples extract with band length 5 mm. Standard Aflatoxin B<sub>1</sub> with various concentrations were also applied in the precoated TLC plate. The TLC plates were spotted with samples in batches and standards were kept in the development chamber filled up with chloroform-acetone (9:1). The solvent were allowed to migrate in the TLC plate. Then plate is air dried. The air dried plate is mounted on Scanner Tray of HPTLC and fixed with the magnets. The spotted plates in TLC scanner were scanned under UV light at 366 nm. The developed analytical method was validated as per ICH guidelines. Table -1 indicate the Method validation parameters and results. Table -2 represents the instrument conditions. Figure -1 indicates the R<sub>f</sub> values of the standard Aflatoxin B1 and analysed Ragi flour sample.

**TABLE-1 INSTRUMENTAL CONDITIONS FOR THE ANALYSIS OF AFLATOXIN B1 IN RAGI FLOUR SAMPLES**

| Description                  | Details  |
|------------------------------|--|
| Element                      | Aflatoxin B1                                       |
| Lamp                         | Hg   |
| Wavelength                   | 366 nm   |
| High voltage (v)             | 182  |
| <b>Application parameter</b> |  |
| Plate Size                   | 20 X 10 cm   |
| Slit Width                   | 6.00 × 0.45 mm, micro                              |
| Starting position            | 15 mm  |
| Band length                  | 8.0 mm   |
| Saturation time              | 5-10 min   |
| Distance space               | 13 mm  |
| Scanning speed               | 20 mm/s  |
| Migration distance           | 70 mm  |
| Mobile Phase                 | Chloroform: Acetone: Water (140:20:0.3)<br>(v/v/v) |

TABLE 2- METHOD VALIDATION PARAMETERS AND RESULTS

| SL. NO. | PARAMETERS                                 | RESULTS     |
|---------|--|-------------|
| 1       | Wavelength (nm)                            | 366         |
| 2       | Retention factor (Rf)                      | 0.76 ± 0.05 |
| 3       | Linearity (ng/spot) (Figure-2)             | 0.25 – 3    |
| 4       | Correlation coefficient ( r <sup>2</sup> ) | 0.99        |
| 5       | Recovery % (Table-3)                       | 77.49       |
| 6       | LOD  | 3.27        |
| 7       | LOQ (matrix)                               | 9.91        |
| 8       | Specificity                                | Specific    |

#### 2.4 CALCULATION:

The concentration of Aflatoxin B<sub>1</sub> in µg/kg has been calculated as follows:

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

Where, B = average Area/Height of Aflatoxin B<sub>1</sub> peaks in test aliquots.

Y = concentration of Aflatoxin B<sub>1</sub> standards, µg/ml

S = µl of Aflatoxin B<sub>1</sub> standards spotted

V = final volume of test solution, µl

Z = average Area/Height of Aflatoxin peaks in standards aliquots.

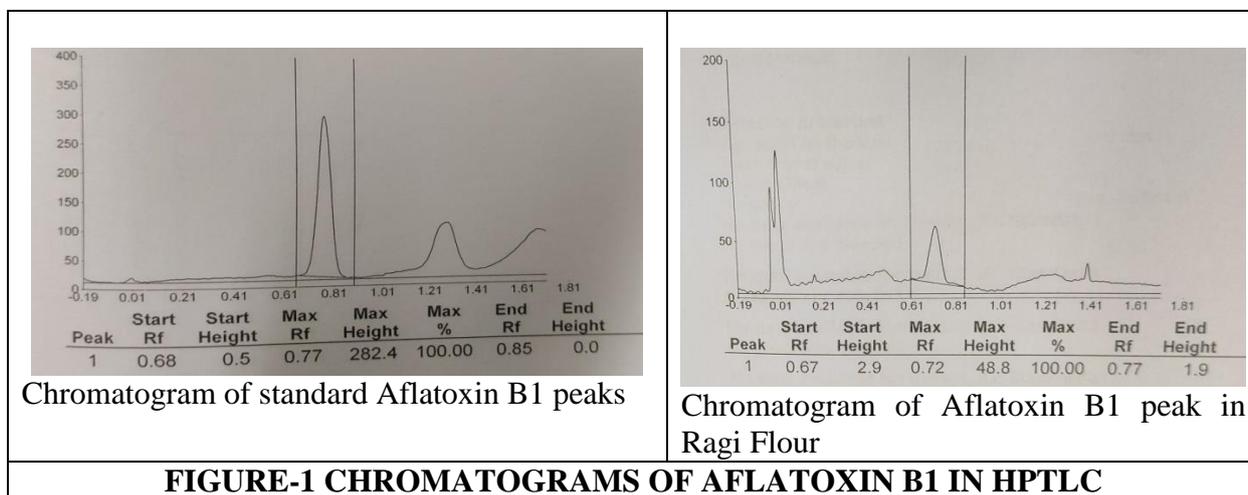
X = µl test solution spotted.

W = g test portion represented by test solution.

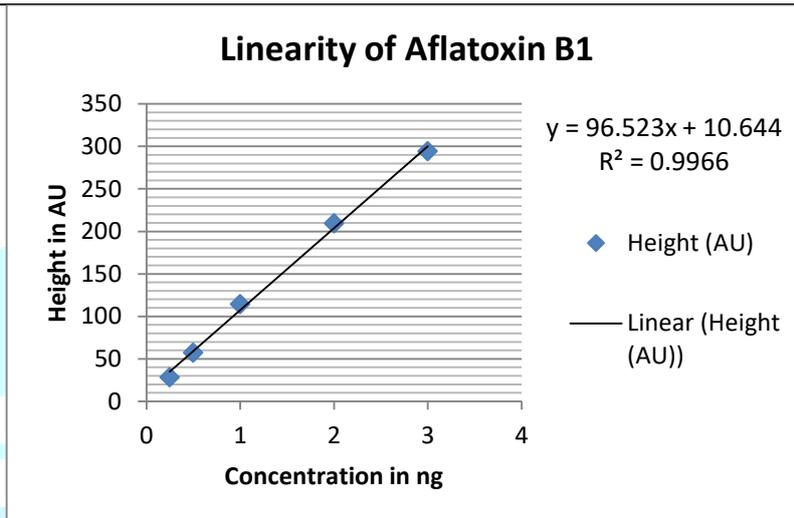
The final results have been obtained by taking average of concentration of Aflatoxin after calculation with respect to Height and Area.

TABLE 3 : RECOVERY PERCENTAGE OF AFLATOXIN B1

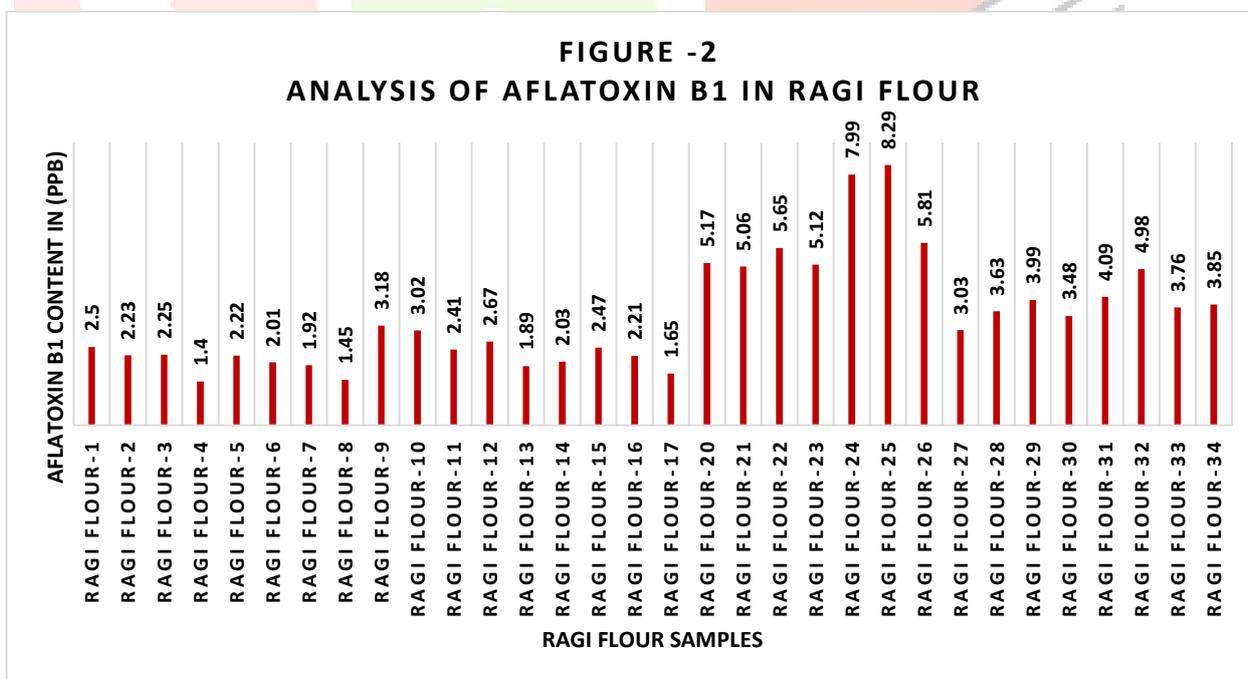
| S. No. | Spike in sample (ppb) | Average Height of spike sample (AU) | Average Height of pure sample (AU) | Height of added spike | Concentration of added Spike from calibration graph (ng) | Weight of sample (g) | concentration of added spike (ppb) | Recovery (%) | Average Recovery | STD Deviation | % RSD   |
|--------|-----------------------|-------------------------------------|------------------------------------|-----------------------|--|----------------------|------------------------------------|--------------|------------------|---------------|---------|
| 1      | 2                     | 23.90                               | 0.00                               | 23.90                 | 0.137331   | 25.0189              | 1.37                               | 68.61        | 77.49            | 8.0809        | 10.4280 |
| 2      | 2                     | 24.21                               | 0.00                               | 24.21                 | 0.140542   | 25.1209              | 1.40                               | 69.93        |                  |               |         |
| 3      | 2                     | 26.52                               | 0.00                               | 26.52                 | 0.164474   | 25.1935              | 1.63                               | 81.61        |                  |               |         |
| 4      | 2                     | 26.09                               | 0.00                               | 26.09                 | 0.160020   | 25.1186              | 1.59                               | 79.63        |                  |               |         |
| 5      | 2                     | 27.73                               | 0.00                               | 27.73                 | 0.177010   | 25.2362              | 1.75                               | 87.68        |                  |               |         |



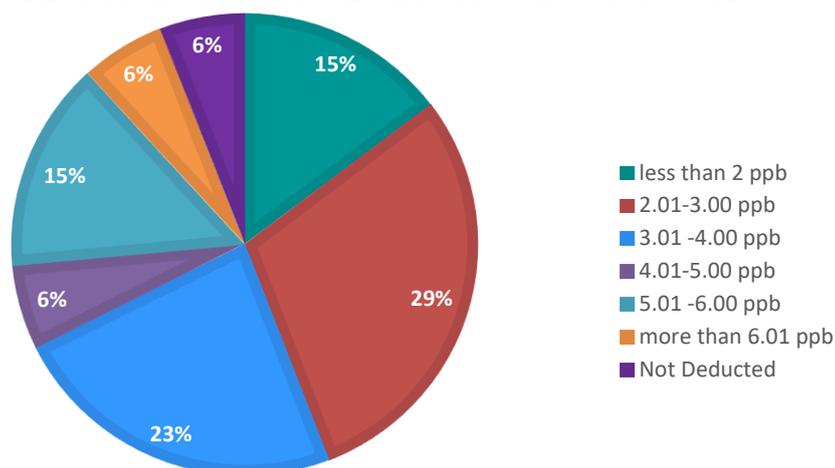
**FIGURE-1 CHROMATOGRAMS OF AFLATOXIN B1 IN HPTLC**



**FIGURE 2: Calibration curve of Aflatoxin B1**



**FIGURE- 3**  
**AFLATOXIN B1 RANGE IN THE ANALYSED RAGI FLOUR SAMPLES**



### 3.RESULT AND DISCUSSION:

A study in 2017 observed that Aflatoxin B1 was not detected in all the millet samples collected during pre-harvesting, harvesting, and postharvesting conditions indicating no natural occurrence, However susceptibility of finger millet samples to aflatoxin production after *A. flavus* infestation was more on 6 and 9 days under controlled conditions in postharvesting (market) conditions [8]. But the study conducted at Malawi during 2025 indicated that fully germinated and fermented finger millet with 1.44 ppb and partially germinated finger millet had a lower contamination level of 0.74 ppb [9]. Aflatoxin contamination occurs more during post-harvest than during pre-harvest conditions [10].

**TABLE- 4 AFLATOXIN B1 CONTENT OF THE RAGI FLOUR SAMPLES**

| SI. No. | Sample        | Aflatoxin B1 content in (ppb) | SI. No. | Sample           | Aflatoxin B1 content in (ppb) |
|---------|---------------|-------------------------------|---------|------------------|-------------------------------|
| 1       | Ragi Flour-1  | 2.50                          | 21      | Ragi Flour-21    | 5.06                          |
| 2       | Ragi Flour-2  | 2.23                          | 22      | Ragi Flour-22    | 5.65                          |
| 3       | Ragi Flour-3  | 2.25                          | 23      | Ragi Flour-23    | 5.12                          |
| 4       | Ragi Flour-4  | 1.40                          | 24      | Ragi Flour-24    | 7.99                          |
| 5       | Ragi Flour-5  | 2.22                          | 25      | Ragi Flour-25    | 8.29                          |
| 6       | Ragi Flour-6  | 2.01                          | 26      | Ragi Flour-26    | 5.81                          |
| 7       | Ragi Flour-7  | 1.92                          | 27      | Ragi Flour-27    | 3.03                          |
| 8       | Ragi Flour-8  | 1.45                          | 28      | Ragi Flour-28    | 3.63                          |
| 9       | Ragi Flour-9  | 3.18                          | 29      | Ragi Flour-29    | 3.99                          |
| 10      | Ragi Flour-10 | 3.02                          | 30      | Ragi Flour-30    | 3.48                          |
| 11      | Ragi Flour-11 | 2.41                          | 31      | Ragi Flour-31    | 4.09                          |
| 12      | Ragi Flour-12 | 2.67                          | 32      | Ragi Flour-32    | 4.98                          |
| 13      | Ragi Flour-13 | 1.89                          | 33      | Ragi Flour-33    | 3.76                          |
| 14      | Ragi Flour-14 | 2.03                          | 34      | Ragi Flour-34    | 3.85                          |
| 15      | Ragi Flour-15 | 2.47                          |         |                  |                               |
| 16      | Ragi Flour-16 | 2.21                          |         |                  |                               |
| 17      | Ragi Flour-17 | 1.65                          |         |                  |                               |
| 18      | Ragi Flour-18 | N.D                           |         |                  |                               |
| 19      | Ragi Flour-19 | N.D                           |         |                  |                               |
| 20      | Ragi Flour-20 | 5.17                          |         | *ND-Not Deducted |                               |

The samples involved in the current study were post harvested and milled ragi samples collected from market from various regions of India. The present study at Central Agmark Laboratory, Nagpur infers that Aflatoxin contamination was more than 7ppb in two samples that 7.99 ppb and 8.29 ppb in the analysed samples (Figure-2). Figure- 3 indicates that 34 ragi flour samples from various regions of India show 15 % of the analysed samples were found to have Aflatoxin B1 less than 2 ppb and a maximum of 29 % of ragi samples had 2.01 to 3.00 ppb of Aflatoxin B1 and 23 % of the samples had a range of 3.01 to 4.00 ppb. Only 2 samples that is 6 % of the analysed samples were not deducted for Aflatoxin B1.

#### 4.CONCLUSION:

In the present study, HPTLC method used to detect the Aflatoxin B1 contamination in the Ragi Flours indicated that the market samples collected from various region of India are well within the prescribed limits of FSSAI and are safe for consumption. 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 (Contaminants, Toxins and Regulations) 2011 as tolerance limit of 30µg/Kg[11] for aflatoxin for all foods meant for human consumption. The EU established maximum levels of AFB<sub>1</sub> and total AFs (2 and 4 µg/kg, respectively) in cereals for human consumption[12].

The authors are intend that the samples collected for the study doesnot possess health risk of Aflatoxin B1 contamination unless of accumulated of toxicity on consumption with other foods.

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