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# Analysis of Maize for Aflatoxin Content from Food Safety and Public Health Perspective

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## ABSTRACT

Epidemiological investigation of prevalence of aflatoxin content in maize was conducted to determine the severity of mold infestation and its possible health impact in human and animal population. Ninety six maize samples were subjected to direct competitive ELISA method and mean was calculated. The determined mean aflatoxin content was 38.77 ppb and found to be very high for human and animal consumption. Results were statistically analyzed for mean, variance, standard deviation and coefficient of variation. Coefficient of variation was narrow and it depicted that irrespective of grown areas aflatoxin content in maize was significantly higher in Central and Northern areas of India.

**Keywords** *Aspergillus flavus, Aspergillus parasiticus,* Parts per billion, ELISA, Optical density.

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## INTRODUCTION

Food grain maize is very prone to mold growth (*Aspergillus flavus and Aspergillus parasiticus*). Fungal metabolite aflatoxin (B1, B2, G1 and G2 ) is a common carcinogen for human and a serious health threat. A study was conducted to determine total aflatoxin content in maize grains. Ninety six samples of maize were collected and subjected to detection by ELISA method. Random sampling method was followed and samples were collected from various maize growing areas of Punjab, Haryana, Uttar Pradesh, MP and Bihar State of Northern and Central India.

## MATERIALS AND METHODS

Five grams of finely grounded sample was mixed with 25 ml of 70% ACS grade methanol (Fisher Scientific) and vigorously shaked with a mechanical vortex shaker for 3 minutes. Each sample was blended for 1 minute in a high speed blender. The mixture was then filtered through Whatman # 1 filter syringe and filtrate was collected in sterile eppendorf tube for analysis. Neogen Veratox total aflatoxin quantitative ELISA (AOAC Licence number 050901) kits were used for detection and quantification of total aflatoxin; 100 µl of each sample were mixed with 100 µl of conjugate in mixing vials using pipettor. Sample conjugate mixture were then transferred to antibody wells and kept for 2 minutes. The antibody wells were washed with 200 µl of deionized water for 5 times using Robonik

**Table 1.** Aflatoxin content in samples in ppb.

| Sample maize | Aflatoxin (ppb) | Sample maize | Aflatoxin (ppb) | Sample maize | Aflatoxin (ppb) | Sample maize | Aflatoxin (ppb) |
|---|---|---|---|---|---|---|---|
| A1 | 43.9 | C1 | 47.2 | E1 | 57.2 | G1 | 48.7 |
| A2 | 39.7 | C2 | 41.5 | E2 | 29.8 | G2 | 32.6 |
| A3 | 48.1 | C3 | 44.3 | E3 | 37.2 | G3 | 39.5 |
| A4 | 54.4 | C4 | 31.9 | E4 | 45.4 | G4 | 33.6 |
| A5 | 41.7 | C5 | 33.6 | E5 | 42.9 | G5 | 40.6 |
| A6 | 49.3 | C6 | 37.2 | E6 | 48.9 | G6 | 46.1 |
| A7 | 34.9 | C7 | 29.7 | E7 | 41.6 | G7 | 36.4 |
| A8 | 38.1 | C8 | 32.6 | E8 | 32.7 | G8 | 26.3 |
| A9 | 51.5 | C9 | 32.4 | E9 | 39.4 | G9 | 29.8 |
| A10 | 32.8 | C10 | 28.6 | E10 | 33.1 | G10 | 34.1 |
| A11 | 44.6 | C11 | 51.3 | E11 | 28.1 | G11 | 30.7 |
| A12 | 31.1 | C12 | 35.4 | E12 | 26.5 | G12 | 32.5 |
| B1 | 36.4 | D1 | 27.6 | F1 | 46.3 | H1 | 52.6 |
| B2 | 35.7 | D2 | 54.8 | F2 | 49.4 | H2 | 44.6 |
| B3 | 43.3 | D3 | 47.3 | F3 | 34.7 | H3 | 30.2 |
| B4 | 30.4 | D4 | 27.9 | F4 | 49.6 | H4 | 25.9 |
| B5 | 35.8 | D5 | 28.4 | F5 | 40.4 | H5 | 34.6 |
| B6 | 39.3 | D6 | 34.8 | F6 | 41.6 | H6 | 38.1 |
| B7 | 37.6 | D7 | 42.9 | F7 | 29.2 | H7 | 39.9 |
| B8 | 35.1 | D8 | 41.7 | F8 | 30.4 | H8 | 46.9 |
| B9 | 36.3 | D9 | 52.9 | F9 | 25.7 | H9 | 50.3 |
| B10 | 40.8 | D10 | 55.6 | F10 | 28.3 | H10 | 51.7 |
| B11 | 41.6 | D11 | 26.8 | F11 | 46.7 | H11 | 38.4 |
| B12 | 45.7 | D12 | 25.2 | F12 | 41.5 | H12 | 31.6 |

washwell ELISA plate washer; 100 µl of substrate were transferred to antibody wells and timer was set for 3 minutes. Remaining substrate was discarded and 100 µl of stop solution were dispensed to each well. Sample plates were read within 5 minutes with TECAN infinity F 50 ELISA plate reader at 650 nm. Optical density values were subjected to Neogen's Veratox software for calculating the results in ppb (parts per billion).

## RESULTS AND DISCUSSION

The mean aflatoxin content in 96 samples of maize was found to be 38.77 ppb. Aflatoxin content of individual sample were enlisted in Tables1and 2.

**Table 2.** Aflatoxin range with number of samples.

| Aflatoxin content range (ppb) | Number of samples | Safety limit <20 ppb |
|---|---|---|
| Between 25–30 ppb | 16 samples | Beyond safety limit |
| Between 30–40 ppb | 39 samples | Beyond safety limit |
| Between 40–50 ppb | 31 samples | Beyond safety limit |
| More than 50 ppb | 10 samples | Beyond safety limit |

Sixteen samples were having aflatoxin content between 25–30 ppb, 39 samples were having between 30–40 ppb, 31 samples were having between 40–50 ppb and 10 samples were having more than 50 ppb. From the results it is observed that all of the 96 analyzed samples having more than the maximum permissible aflatoxin action level of 20 ppb established by food and drug administration (FDA). The results reveal a serious health issue due to high aflatoxin content in maize in Northern and Central India.

## CONCLUSION

Variance of the samples was 66.40, standard deviation was 8.15 and coefficient of variation was 21.02% as calculated from statistical analysis. Standard deviation obtained was less than 10 whereas sample mean was high at 38.77 ppb; the result depicted that irrespective of samples obtained from different agro climatic region of North and Central India aflatoxin content was higher than the recommended level of 20 ppb. Narrow coefficient of variation of 21.02% indicated the widespread severity of aflatoxin con-

tamination when the sample mean was significantly higher than recommended action level of FDA.

Aflatoxins are potent carcinogen, mutagenic and genotoxic in pathogenic bacteria (WHO, Department of Food Safety and Zoonoses, February 2018). The potency of aflatoxin to cause liver cancer is significantly enhanced in presence of immunosuppression (e.g. tuberculosis, HIV) and in presence of infection with Hepatitis B Virus (WHO, February 2017, 2018). Tuberculosis and Hepatitis B infections are inherent problems of Indian health care system from public health perspective as remedial measures are lengthy and limiting. It is therefore concluded from the results of the study that higher aflatoxin level in food grain maize is a serious public health concern.

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