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## RP Senthilkumar

Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

## A Natarajan

Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

## R Kavitha

Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

## Corresponding Author:

### RP Senthilkumar

Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

## Co-occurrence of mycotoxins in pearl millet

RP Senthilkumar, A Natarajan and R Kavitha

### Abstract

A study was undertaken to assess the mycotoxins occurrences in pearl millet (Bajra). A total of 121 samples of pearl millet samples received from all over the country were subjected to semi quantitative multimycotoxin method for the determination of aflatoxin (B1, B2, G1 and G2), ochratoxin A, citrinin and T2-toxin. Out of the samples tested, about 52.07% samples were found to be contaminated with any one of the mycotoxins tested. Of the mycotoxin tested, aflatoxin B1 is the predominate one with 45.45% occurrence followed by aflatoxin B2 (28.93%). Ochratoxin A (OTA) and Citrinin were detected in 8.16% and 7.14% samples. Further, 7.14% of the samples found to have co-occurrence of more than one mycotoxins viz., aflatoxin, ochratoxin A and Citrinin. Maximum concentration of AflatoxinB1, B2, ochratoxin A and citrinin are detected at 150, 40, 405 and 2070 µg/kg respectively. The study concludes that co-occurrence of mycotoxins in pearl millet.

**Keywords:** sunflower meal, mycotoxins, aflatoxin, ochratoxin

### Introduction

Increasing cost of maize has become a major bottleneck for the profit margins in poultry production. Nutritive and feeding value of pearl millet as alternative, locally available energy sources has been studied well and can form 100% replacement of maize in poultry feed. Pearl millet is being fed to backyard chicken as supplementary feed in Tamil Nadu. However, use of maize in poultry poses threat due to the presence of high level of mycotoxins at times. In a survey program monitoring the worldwide prevalence and concentration of various mycotoxins in feed and feed raw materials, Streit (2013) [9] revealed that a total number of 139 different fungal metabolites were detected in the 83 feed samples and all of the samples were co-contaminated with seven to 69 different mycotoxins. Hence, a study was undertaken to assess the mycotoxins occurrence in pearl millet in India.

### Materials and Methods

Occurrence of mycotoxins in pearl millet was investigated between April 2017 to March 2018 at Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal, Tamil Nadu. A total of 121 pearl millet samples received from all over the country were subjected to analyses of various mycotoxins such as aflatoxin (B1, B2, G1 and G2), ochratoxin A, citrinin and T2 toxin by two dimensional thin layer chromatographic methods as suggested by Tapia (1985) [10]. In brief, the toxin was extracted with acetonitrile, potassium chloride in hydrochloric acid, filtered and defatted with hexane twice. The fat free extract is further extracted by chloroform, dried and re-diluted with chloroform and spotted on pre-coated (0.25 mm) silica gel G on alumina sheet (Merck) along with known standards. The plates were developed in chloroform and acetone on one direction and toluene: ethyl acetate: formic acid in second direction perpendicular to the first. The plates are dried and quantified under exposure in long wave UV light and the toxin concentration is expressed in parts per billion (µg kg<sup>-1</sup>). The mycotoxins were further confirmed by spraying/exposing the plates with diluted sulphuric acid, aluminium chloride solutions and ammonium hydroxide. The levels of mycotoxins so determined in pearl millet were subjected to descriptive statistical analysis (Steel *et al.*, 1996) [8].

### Results and Discussion

Occurrences of aflatoxins, ochratoxin A, citrinin and T2 toxin in pearl millet are presented in Table 1 and 2.

Tested pearl millet samples were found to contain aflatoxin (B1 and B2), and ochratoxin A and citrinin at various concentrations.

The percentage of occurrence of aflatoxin and ochratoxin were decreasing at increasing concentration levels of the toxins. Out of 121 pearl millet samples tested, 52.07% of the samples had aflatoxin B1. In addition, out of 98 samples tested for ochratoxin A and citrinin, co occurrence of mycotoxins were detected in 7.14% of the samples. The data suggest that the type of fungal infestation and associated mycotoxins production in pearl millet is similar to that of maize and ground nut meal (Senthilkumar *et. al.*, 2012) [6]. Ranges of occurrence of mycotoxin in pearl millet are presented in table 2. Out of mycotoxins tested, aflatoxin B1 occurrence is at maximum of 63% of the samples. About 46% samples have Aflatoxin B1 concentration upto 50 µg/kg whereas, concentration at 50-100 and 101-500 µg/kg are detected each in 7% of the samples.

Co-occurrence of Aflatoxin B2 was detected along with aflatoxin B1 at 28.93% samples while none of the samples contains aflatoxin B2 alone. Similar to aflatoxin B1, occurrence of aflatoxin B2 is decreasing as the concentration increases. Similar observation is also reported by Ragavendra *et al* (2007). Maximum concentration of aflatoxin B1 and B2 are not exceeded 150 and µg/kg in the sample. This level of aflatoxin B1 and B2 indicates that the concentration of these toxins is relatively at lower level when compared to maximum concentration recorded in maize and groundnut meal (Senthilkumar *et. al.*, 2012) [6]. Unlike that of maize and groundnut meal, aflatoxin G1 and G2 were not detected in the tested samples.

Field cases of ochratoxicosis in broilers and layers with adverse effect on their performance have been reported (Page *et al.*, 1980 and Doerr *et al.*, 1981) [5, 3]. In the present study, ochratoxin A (OTA) was detected in 8.16% of the samples analysed and at the concentration falls majorly from 12 – 50 µg/kg. Less number of samples (1-2%) found to contain ochratoxin A at higher levels and a maximum concentration

detected at 405 µg/kg. These data suggests that though occurrence of OTA was less than aflatoxin B1, the concentration will be higher than aflatoxin B1 at times. Citrinin was detected in 7% of the samples and the concentration is at maximum of 2070 µg/kg. The concentration and occurrence of citrinin follows different pattern when compared to aflatoxin, as the number of sample increases at higher concentration of citrinin. However, number of samples with high occurrence of citrinin is limited in this study. Egyptian maize reported to have occurrence of citrinin at 15% level (Abd hul Hameedh, 1990). Sarathchandra and Muralimanohar (2013) reported that feed and maize had citrinin at a concentration ranging from 20-350 µg/kg. The observation in the present study is in accordance with the above findings.

Simultaneous or co-occurrence of more than one mycotoxin was reported to be more toxic than presence of one mycotoxin (Klaric *et. al.* 2013 and Clarke *et. al.* 2014) [4, 2]. Irrespective of mycotoxins tested, the co-occurrences of mycotoxins were high at their lower concentration levels and the co-occurrence decreased while concentration of mycotoxins increases. As demonstrated by Stec *et. al.* (2009) [7], aflatoxin and ochratoxin are strong immune-suppressive toxins than citrinin. Hence, co-occurrence of both aflatoxin and ochratoxin in poultry feed ingredients may lead to possible immune-suppression.

In conclusion, about 52, 7.14 and 8.16 per cent of the pearl millet samples had occurrence of aflatoxin B1, citrinin and ochratoxin respectively. Maximum concentration of Aflatoxin B1, ochratoxin A and citrinin are detected at 150, 405 and 2070 µg/kg respectively. None of the samples tested have aflatoxin G1 and G2. There is co-occurrence of mycotoxin at least about 7.14% of the samples. Hence, continuous monitoring of multiple mycotoxin in pearl millet is essential as measure of quality control in feed processing.

**Table 1:** Percentage occurrence/co-occurrence of different mycotoxins at various levels in pearl millet.

Samples	Toxin free samples	Infested samples	Occurrence of toxins	
			Aflatoxin	Other toxins
No. of samples	58	63	55	8
Percentage	47.93	52.07	45.45	8.16

**Table 2:** Occurrence of mycotoxin and its levels in pearl millet

Name of the toxin	Levels of toxins (µg/kg)					
	Negative	Positive	≤50	51 – 100	101 - 500	> 500
No of samples and percentage						
Aflatoxin B1	58 (47.93)	63 (52.07)	46 (38.01)	9 (7.44)	9 (7.44)	0
Aflatoxin B2	86 (71.07)	35 (28.93)	31 (14.62)	4 (3.30)	1 (0.83)	0
Aflatoxin G1	100	0	0	0	0	0
Aflatoxin G2	100	0	0	0	0	0
Citrinin	91 (92.86)	7 (7.14)	0	1 (1.02)	2 (2.04)	4 (4.08)
Ochratoxin A	90 (91.84)	8 (8.16)	5 (5.10)	1 (1.02)	2 (2.04)	0

**Note:** Figures in parenthesis indicates actual number of samples.

\*parts per billion = µg/kg

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