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High Incidence of Aflatoxin B_1 in Pearl Millet Samples: A Report

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ABSTRACT

A multi mycotoxin screening report of about 33 pearl millet *(Pennisetum glaucum)* samples were prepared in the Animal Feed Analytical and Quality Assurance Laboratory, Namakkal during the months of August and September 2023. The samples were received to the laboratory from the livestock and poultry farmers of different geographical regions of Tamil Nadu. Among the 33pearl millet samples, the Aflatoxin B_1 was the most predominant toxin, the concentration of Aflatoxin B_1 and B_2 were ranged widely from 11 to 1155µg/kg and 8 to 116µg/kg. The mean value of Aflatoxin B_1 and B_2 observed to be 310.88 ± 57.16µg/kg and 33.71± 5.02µg/kg. The moisture percentage of the pearl millet samples varied from 11.02 to 13.60%. The mean value of the moisture content was 12.07 ± 0.10%. The samples with high Aflatoxin B_1 and high moisture had an earthy odour also. About 57.58 % of the pearl millet samples recorded above100µg/kg for Aflatoxin B_1. Conclusively, the mycotoxin contamination of the samples may be due to moistening and mold growth. The ingestion of high Aflatoxin B_1 contaminated samples by livestock and poultry may result in immune suppression, reduction in egg production, poor weight gain and even mortality also. Hence, the farmers are advised to routinely analyze their feed ingredients like cereals and oilcakes for multi mycotoxins at the nearby feed analytical laboratories to avoid financial loss.

KEYWORDS: Aflatoxin B_1, Feed, Feed ingredients, Livestock, Pearl millet (Bajra), Poultry

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INTRODUCTION

Mycotoxins are the naturally arising toxins formed by certain fungal species like *Aspergillus, Fusarium, Penicillium, Claviceps, Alternaria* and many more (Haque, 2020). Among these, *Aspergillus spp. (flavus and parasiticus)* produces the most predominant aflatoxins which belong to the difurocoumarocyclopentenone group (AFB_1, AFB_2, AFM_1, and AFM_2) and difurocoumarolactone group (AFG_1 and AFG_2) (Ismail, 2018). AFM_1 and AFM_2 are derived from the metabolism of AFB_1 and AFB_2 in the liver of cattle by cytochrome P_{450} and excreted in the milk and the carry-over efficiency in milk varies from one animal to another (Huiying Li, et. al., 2019 and Van Egmond et. al., 2001). The AFB_1 contamination in poultry feed causes immunosuppression, reduced feed intake and compromised the growth and production in broiler and layer chicken (Denli, 2009) but ducks are very sensitive than other fowls.

In tropical areas, feed commodities like cereals and oilcakes are found to be often contaminated, mostly with AFB_1. Cereals pose biggest threat in contributing to the pool of AFB_1 in the finished feeds. To assure the quality of feeds to maintain AFB_1 level within the stipulated limit of 20 ppb (IS 1374 : 2007), stringent measures of quality control and quality assurance are taken by feed manufacturers. Animal Feed Analytical and Quality Control Laboratory (AFAQAL) receives huge number of raw ingredients and records considerable presence of AFB_1 in maize, de-oiled groundnut cake, sunflower meal and certain other feed ingredients. During the later quarter of 2023, thirty-three pearl millet *(Pennisetum glaucum)* samples, received by this laboratory within short span of period, were showing very high AFB_1 contamination and the same is reported and discussed in this article. The purpose of this reporting is to highlight the incidence and to sensitize the feed manufacturers on the need of routine and

planned analytical schedule to avoid high mycotoxins in the finished feeds.

MATERIALS AND METHODS

Sample details and periodicity

A total of 33 pearl millet samples were received from livestock and poultry farmers of Tamil Nadu between 19th August and 21st September 2023, for mycotoxin analysis, as part of their routine preventive measures before inclusion in the compounded feeds. The laboratory has the proficiency of various mycotoxins including AFB_1, AFB_2, AFG_1, AFG_2, Citrinin, Ochratoxin A in feeds and feed ingredients by TLC method and routinely participates in the Proficiency Testing (PT) for AFB_1 since 2015 conducted by Chemistry Department, Texas A & M University with Z score of < 2.0.

Mycotoxin analysis

Multi mycotoxin Estimation

The samples of pearl millet were subjected to analyses of various mycotoxins including aflatoxins (B_1, B_2, G_1 and G_2), by modified two dimensional thin layer chromatographic method (Tapia, 1985). Standard aflatoxin B_1 and B_2 solution was prepared (AOAC 1995). Toxin was extracted with acetonitrile, potassium chloride in hydrochloric acid, filtered and defatted with hexane twice. The fat free extract was further extracted by chloroform, dried and re-diluted with known volume of chloroform and aliquots were 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 were dried and quantified under exposure in long wave UV light and the toxin concentration is expressed in parts per billion ($\mu g/kg^{-1}$)(Senthilkumar et al., 2021). The concentrations of aflatoxins (B_1 and B_2) determined so far in pearl millet was subjected to descriptive statistical analysis (Steel et al., 1996).

Determination of moisture

Moisture content in the pearl millet samples was determined by oven method (AOAC, 2011) and expressed in per cent on as such basis.

RESULTS AND DISCUSSION

AFAQAL received thirty three pearl millet samples for multi mycotoxin analysis in a short span of time (August 2023 – September 2023) from feed millers of Tamil Nadu. Upon interrogation, it was understood that all these samples originated from the same lot which arrived in rail wagons from other part of the country for distribution to feed millers.

Content of aflatoxin B_1, B_2, citrinin, ochratoxin A and moisture in all the thirty three pearl millet samples is presented (Table. 1) of which, 32 samples were found positive for AFB_1 and AFB_2 but all were negative for Citrinin. However, ochratoxin A was found in one pearl millet sample with a concentration of 585Øµßg⁄kg.

The concentration of AFB_1 and AFB_2 in the pearl millet samples (Table. 1) has been categorized into the levels of negative (below LoQ of 5µg/kg), < 20, 21 – 50, 51 – 100, 101 – 500, 501 – 1000 and > 1000µg/kg. Pearl millet samples were found to be contaminated with AFB_1 between 11 and 1155µg/kg while one sample was found to be negative for AFB_1 and another one with <20µg/kg. Rest of the pearl millet samples generally contained alarming levels of AFB_1, with 19 samples (57.58 %) showing very high levels of AFB_1 (>100µg/kg). While 11 samples (33.33 %) had AFB_1 between 101 – 500µg/kg, 6 samples (18.18 %) showed between 501 and 1000µg/kg and two samples had > 1000µg/kg.

Table. 1. Percentage of Aflatoxins (µg/kg) occurred at various levels in Bajra.

	Level of Aflatoxin (µg/kg)						
	Nil	<20	21 – 50	51 – 100	101 – 500	501 – 1000	> 1000
Aflatoxin B_1	1	1	4	8	11	6	2
Percentage (%)	3.03	3.03	12.1	24.2	33.3	18.1	6.06
Aflatoxin B_2	2	13	9	8	1	-	-
Percentage (%)	6.06	39.39	27.2	24.2	3.03	-	-

The mean value of AFB_1 infestation in these pearl millet samples was 310.88 ± 57.16µg/kg. This level of contamination (310.88 ± 57.16µg/kg) of AFB_1 can easily harm any livestock and poultry, based on the inclusion level in the feed, leading to immunity problems and poor production performances. The aflatoxin contamination of the pearl millet samples could be due to the factors like grain moisture, environmental temperature, relative humidity, presence of light and even pH (Kumar et. al., 2021 and Yoshinari et. al., 2010), apart from a possible factor of a rain during harvest or post-harvest.

Hence, all the samples were analysed for moisture to assess whether high moisture could be a reason for the infestation of AFB_1 and other mycotoxins. The results are presented in the Table 2.

Table 2. The content of different mycotoxins (µg/kg) in the pearl millet samples received byAFAQAL, Tamil Nadu, between August 2023 - September 2023.

S.No	Moisture (%)	Aflatoxin B_1	Aflatoxin B_2	Citrinin	Ochratoxin A
1	13.3	1155	87	ND	ND
2	11.02	61	10	ND	ND
3	12.3	116	14	ND	ND
4	12.08	462	58	ND	ND
5	12.4	347	43	ND	ND
6	11.1	347	43	ND	ND
7	13.04	924	116	ND	ND
8	12.7	690	87	ND	ND
9	11.9	462	43	ND	ND
10	12.9	44	11	ND	ND
11	13.05	750	63	ND	ND
12	12.6	1001	78	ND	ND
13	11.7	22	8	ND	ND
14	12.3	156	16	ND	ND
15	12.9	66	16	ND	ND
16	12.9	66	11	ND	ND
17	12.8	188	23	ND	ND
18	12.4	188	22	ND	58
19	12.5	375	63	ND	ND
20	12.6	250	46	ND	ND
21	12.8	ND	ND	ND	ND
22	12.7	63	8	ND	ND
23	13.6	876	31	ND	ND
24	12.6	11	-	ND	ND
25	12.2	55	11	ND	ND
26	12.7	66	16	ND	ND
27	13.3	625	63	ND	ND
28	12.1	375	63	ND	ND
29	11.9	55	11	ND	ND
30	12.1	625	47	ND	ND
31	11.3	27	8	ND	ND
32	11.7	78	8	ND	ND
33	12.0	44	22	ND	ND
Mean ± SE	12.07 ± 0.10	310.88 ± 57.16	33.71± 5.02	-	-
Range	11.02 – 13.60	11 – 1155	08 – 116	-	-

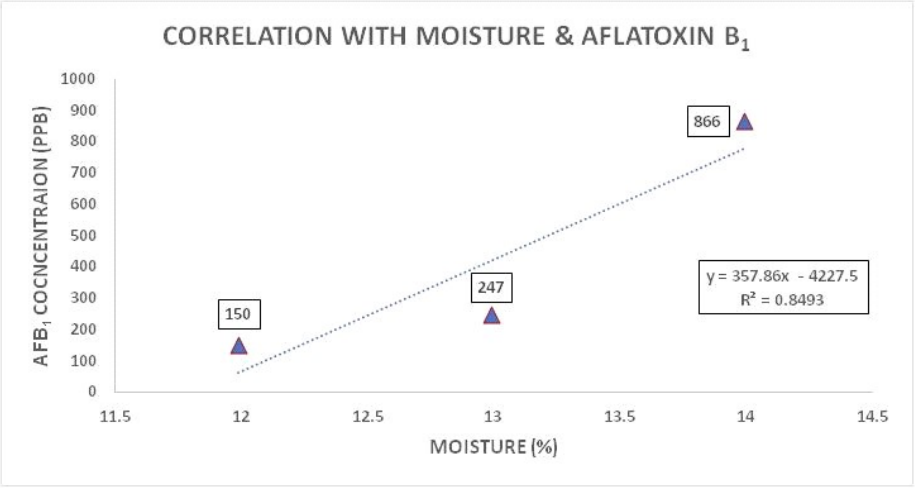
*ND – Not Detected

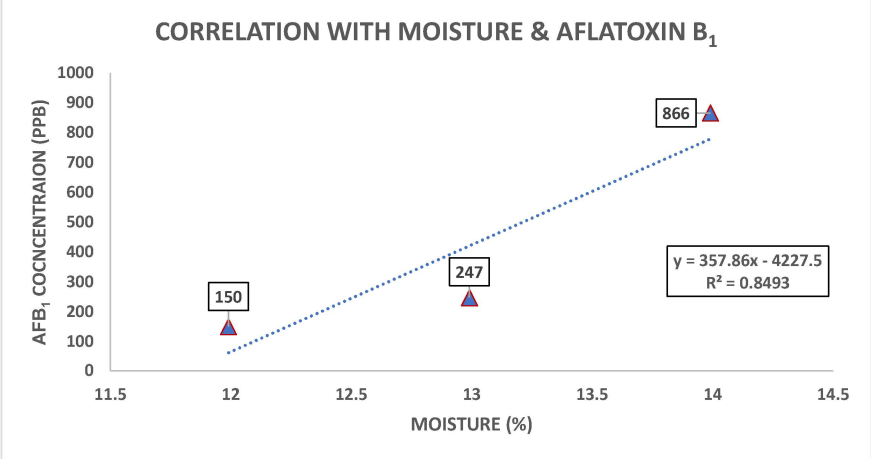
These samples were found to contain high moisture (mean – 12.07 ± 0.10 %, range – 11.02 – 13.60 %). Upon categorization of grains into three levels of moistures i.e. 11.00 – 11.99 %, 12.00 – 12.99 % and 13.00 – 13.99 %, the corresponding mean AFB_1 content revealed a striking relationship between moisture content and AFB_1 content (Table. 3). This strengthened the thought that, moisture might be the reason for AFB_1 and other mycotoxins in the pearl millet samples. It also implies that the entire lot of pearl millet could have had high moisture content before they were loaded at the starting point. Further, there existed a strong relationship between moisture and the AFB_1 contents (µg/kg) that were fit in a graph (Fig. 1) with a high R^2 (0.849) which consolidated the relationship further between moisture and AFB_1 level. With moisture level went up, the AFB_1 content was also found to be higher (11.00 – 11.99 %, 7 samples, mean = 150µg/kg; 12.00 – 12.99 %, 21 samples; mean = 247µg/kg and 13.00 – 13.99 %, 5 samples, mean = 866µg/kg). High moisture content was an important factor for the fungal growth and release of Aflatoxin B_1 (Achaglinkame et al., 2017) and in rainy season, the incidence of AFB_1 was higherin grains (Raghavender et. al., 2007 and Anjum et. al., 2012).

Table. 3. Correlation of moisture (%) content with Aflatoxin B_1 (µg/kg) in the pearl millet samples

Moisture (%)	No. of Samples with AFB_1	Range (µg/kg)	Mean
11.0 – 11.9	7	22 – 462	150
12.0 – 12.9	21	11 – 1001	247
13.0 – 13.9	5	625 – 1155	866

Figure 1: Correlation of moisture (%) with Aflatoxin B1 (ppb) in the pearl millet samples





Aflatoxin B_2 contamination of the pearl millet samples ranged between 8 – 116µg/kg with a mean value of 33.71± 5.02µg/kg. 31 out 33 (93.94 %) pearl millet samples showed cooccurrence of AFB_2 except in one sample, in which AFB_1 was only 11µg/kg. AFB_2 co-occurred mostly with AFB_1 in the earlier reports also (Senthilkumar et al., 2021 and Raghavender et al., 2007).

Other toxins like Citrinin and Ochratoxin A were not detected in the pearl millet samples tested; however one sample was positive for Ochratoxin A with the concentration of 58µg/kg, which co-occurred with AFB_1 (188µg/kg) and AFB_2 (22µg/kg). This level of ochratoxin could cause deleterious effects for poultry based on the inclusion level of the contaminated material in the feed.

Physical characteristics of pearl millet samples received by AFAQAL during the reported period

Visual characteristics

A pearl millet sample (Fig. 2), meant for human consumption, was purchased from the market and is displayed for comparison with the pearl millet sample that arrived in rail wagon (Fig. 3). The market purchased pearl millet sample was visibly bright, unbroken, greenish yellow and grey in colour and contained 11.28 % of moisture with AFB_1 of 8µg/kg. The wagon-arrived sample was slightly broken,appeared mouldy, and is dark brown, black or yellowish in colour.

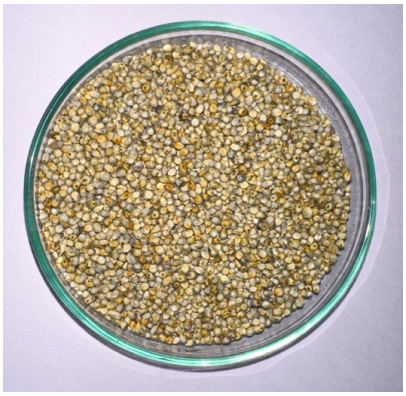


Figure 2: Pearl millet (Bajra) with 8µg/kg of AFB_1

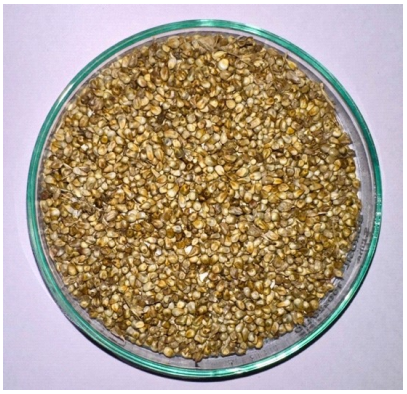


Figure 3: Pearl millet (Bajra) with 1001µg/kg of AFB_1

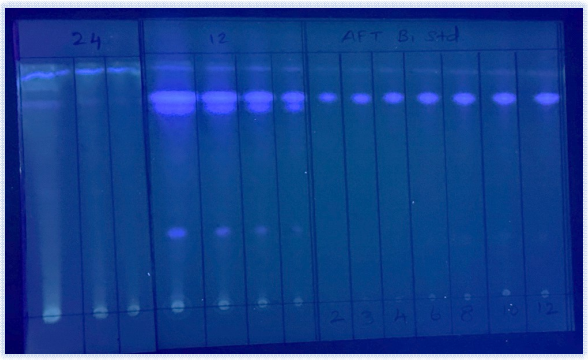


Figure 4: TLC plate showing 8µg/kg of AFB_1 and 1001µg/kg of AFB_1 in Pearl millet (Bajra)

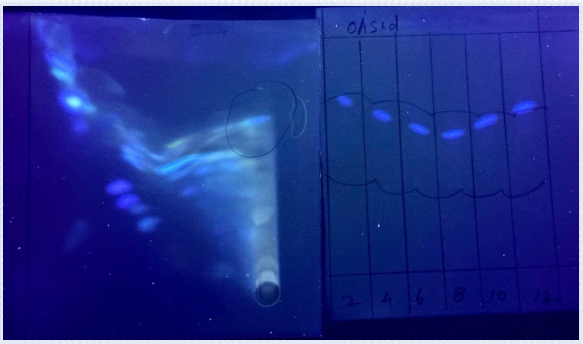


Figure 5: TLC plate showing 58µg/kg of Ochratoxin A in Pearl millet (Bajra)

Odour characteristics

The wagon-arrived samples possessed earthy or muddy odour which increased in intensity with increase in AFB_1 contamination. It could be assumed that the presence of strong earthy or muddy odour may be due to the presence of increased moisture content which would have predisposed to fungal infestation. The market sample was fresh and did not emanate any earthy or muddy odour but with a fresh grainy odour.

CONCLUSION

Pearl millet samples that arrived by wagon to Namakkal had higher moisture (11.02 to 13.60%) and were found to be contaminated with aflatoxin B_1 severely which ranged from 11 to 1155µg/kg. More than half of the pearl millet samples (57.58 %) contained more than 100µg/kg. These contaminated pearl millet samples, upon categorization into three moisture levels, confirmed a strong relationship between moisture and AFB_1 content (R^2 - 0.849). All the pearl millet samples that were infested with AFB_1 and AFB_2 were found to be mouldy with earthy odour and appeared dark brown, black or yellowish in colour. These findings of high AFB_1 in pearl millet samples that arrived by wagon covering long distance suggests that feed manufacturers and millers should be aware of differences in the physical appearance of such infested samples followed by laboratory testing of mycotoxins before accepting for feed manufacturing.

REFERENCES

Achaglinkame, Matthew, Opoku, Nelson and Amagloh, Francis. 2017. Aflatoxin contamination in cereals and legumes to reconsider usage as complementary food ingredients for Ghanaian infants: A review. Journal of Nutrition & Intermediary Metabolism. 10, 1-7.

Anjum, M. A., Khan, S. H., Sahota, A. W. and Sardar, R. 2012. Assessment of Aflatoxin B1 in commercial poultry feed and feed ingredients. The Journal of Animal & Plant Sciences. 22(2): 268 - 272.

AOAC. 1995. Official methods of analysis of the association of official analytical chemists, 16th Edn. Method 971.22; 985.17. The Association, Arlington, Washington.

AOAC. 2011. Official methods of analytical chemist.18th Edn. Gaithersburg (MA): AOAC International. Pp. 100-105.

Denli, M., Blandon, J. C., Guynot, M. E., Salado, S. and Perez, J. F. 2009. Effects of dietary AflaDetox on performance, serum biochemistry, histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B1. Poultry Science 88:1444–1451.

Haque, Yihui Wang, Zhiqiang Shen, Xiaohui Li, Muhammad Kashif Saleemi and Cheng He. 2020. Mycotoxin contamination and control strategy in human, domestic animal and poultry: A review, Microbial Pathogenesis 142, 104095.

Huiying Li, Songli Li, Huaigu Yang, Yizhen Wang, Jiaqi Wang and Nan Zheng. 2019. L-Proline Alleviates Kidney Injury Caused by AFB1 and AFM1 through Regulating Excessive Apoptosis of Kidney Cells. Toxins. 11: 226.

Ismail, A., Bruna, L., Gonçalves, Diane V. de Neeff, Bárbara Ponzilacqua, Carolina F.S.C. Coppa, Henning Hintzsche, Muhammad Sajid, Adriano G. Cruz, Carlos H. Corassin and Carlos A.F. Oliveira, 2018. Aflatoxin in foodstuffs: Occurrence and recent advances in decontamination. Food Research International. 113: 74-85, ISSN 0963-9969.

Raghavender et al., 2007. Aflatoxin contamination of pearl millet during field and storage conditions with reference to stage of grain maturation and insect damage. Mycotoxin Research. 23(4): 199-209.

Senthilkumar et al., 2021. Co-occurrence of mycotoxins in pearl millet. The Pharma Innovation Journal. 10(3): 253-255.

Steel, R.G., Torrie, J.H. and Dickey,D.A. 1996. Principles and Procedures of Statistics. A Biometrical Approach. 3rd Edn. McGraw-Hill Companies, New York.

Tapia, M.O. 1985. A quantitative thin layer chromatography method for the analysis of aflatoxins, ochratoxin A, zearalenone, T-2 toxin and sterigmatocystin in foodstuffs. Revista Argentina de Microbiologia. 17(4):183-186. PMID: 2961006.

Van Egmond, H.P. and Dragacci, S. 2001. Liquid chromatographic method for aflatoxin M1 in milk. Methods Molecular Biology. 157: 59–69.

Yoshinari, T., Noda, Y., Yoda, K., Sezaki, H., Nagasawa, H., and Sakuda, S. 2010. Inhibitory activity of blasticidin a, a strong aflatoxin production inhibitor, on protein synthesis of yeast: Selective inhibition of aflatoxin production by protein synthesis inhibitors. The Journal of Antibiotics. 63(6):309–314.