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Detection of Aflatoxin B_1 through indirect-ELISA from fresh grains obtained from three maize growing zones of India

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Abstract: Aflatoxin B_1 (AFB_1) is most frequently found in plant substrates, which has shown the highest toxigenic potential. Based on previous studies, the IARC has classified AFB_1 as a class 1A human carcinogen. Several impacts on consumers, such as loss of human and animal lives; health care and veterinary care costs; contaminated foods and feeds disposal costs; and investment in research and management of the myco-toxin problem. Fourteen maize seed samples comprising of recommended and local varieties were collected from three maize growing zones (Zone I- Almora, Kullu, Bilaspur, Dhaulakuan, Kangara, Saharanpur, Zone II- Karnal, Ludhiana, Pantnagar, New Delhi and Zone III- Begusarai, Varanasi, Sabour-1 and Sabour 2). In our studies AFB_1 toxin range were noticed Zone-I (0.0294- 153.5081 ppb), Zone-II (0.1761- 161.0537 ppb ppb) and Zone-III (3.8366- 53.1256 ppb) collected seed samples.This indicate that ELISA technique could be applied to the monitoring of Aflatoxin contamination in a lot of samples in a cost, accuracy, simplicity and time effective manner.

Keywords: AFB_1 toxin, *Aspergillus flavus*, Indirect-ELISA, Maize

INTRODUCTION

The naturally toxigenic fungal flora, existing in conjunction with food production is most dominated genera namely, *Aspergillus, Fusarium* and *Penicillium* and lesser extent the *Alternaria, Claviceps* and *Stachybotrys*. More than 300 mycotoxins are known to exist in nature. The economically important species of fungi producing significant mycotoxins as: Aflatoxins (AFs), citrinin (CIT), cyclopiazonic acid (CPA), fumonisins (FBs), moniliformin (MON), ochratoxin A (OTA), deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), patulin (PAT) and zearalenone (ZEA). Aflatoxin B_1 (AFB_1) is most frequently found in plant substrates, which has shown the highest toxigenic potential (Moss, 1998). Based on previous studies, the IARC (1993) has classified AFB_1 as a class 1A human carcinogen (Rothschild, 1992). Several impacts on consumers, such as loss of human and animal lives; health care and veterinary care costs; contaminated foods and feeds disposal costs; and investment in research and management of the myco-toxin problem. Myco-toxins are able to induce powerful and diverse biological effects. Diverse actions of myco-toxins have been characterized on animals and humans to include cytotoxic, carcinogenic, immunosuppressive, nephrotoxic, neurotoxic, mutagenic and oestrogenic effects. Pre and post-harvest management strategies are most important for management of toxicogenic fungi in food materials (Kumar *et al.,* 2015).

In recent years, enzyme linked immunosorbent assays (ELISA) have been described for Aflatoxin determination. ELISA methods potentially have advantages over the other procedures because of their simplicity, sensitivity, low cost and the use of safe reagents. Extensive studies on Aflatoxins in foods have validated ELISA, in comparison with very accurate, but expensive, low throughput research-oriented techniques, such as HPLC and LC/MS (Chun *et al*., 2007, Ayejuyo *et al.,* 2011, Oplatowska-Stachowiak *et al*., 2016).

MATERIALS AND METHODS

Fourteen maize seed samples comprising of recommended and local varieties were collected from three maize growing zones (Zone I- Almora, Kullu, Bilaspur, Dhaulakuan, Kangara, Saharanpur, Zone II- Karnal, Ludhiana, Pantnagar, New Delhi and Zone III- Begusarai, Varanasi, Sabour-1 and Sabour 2). ELISA technique applied to the monitoring of AFB_1 contamination in the samples given by Abbas *et al.* (2004).

Extraction of sample: Weigh 25g ground sample with 2.5g salt (KCl) and place in blender jar. Add to jar 100 ml Methanol: Water (70:30). Cover Blender jar and blend at high speed for 1 minute. Remove cover from jar and pour extract into conical flask. Cover conical flask was placed in shaker for one and half hour. Filtrate was collected by the help of Whatman filter paper (No. 41) in a clean vessel.

ELISA method: AFB_1-BSA carbonate buffer conjugate (150 µl) was added in 66 wells of ELISA plate for

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coating, incubated for 1 hour at 37 °C. After washing 200 µl of BSA 0.2 % was added to each well and incubated again. The healthy maize grain extract diluents were prepared in BSA in 1:9 ratios (Sample 1) and 50 ml of this sample was added in 5 µl of AFB1 standard (1mg/ml) to make it 100 ppb (Sample 2). 100 µl of sample 1 was added to the well B3 to B11 and C3 to C11. 100 µl of standard (Sample 2) was added to the wells B2, B3, C2 and C3. B3 and C3 to become 100 ppb, transfer 100 µl of B3 solution to B4 for serial dilution. The samples were added @10 µl in each well. 50 µl of antiserum (1:6000) were added in each well and incubated at 37 °C for 1 hour followed by 3 washing. 150 µl Antiserum diluents (1:4000) were added in each well followed by incubation then washing. 150 µl of substrate buffer for alkaline phosphatase were added to each well and kept for incubation.

Colour change was observed in each well from colorless to yellow this yellow color shows the presence of Aflatoxin. Absorbance was measured at 405 nm in an ELISA reader (Thermo Multiscan EX) using the values obtained for AFB1 standards draw a curve using with the help of a software sigma plot, taking log of Aflatoxin concentrations on the X-axis and optical density values on the Y- axis and calculate AFB_1 in ppb.

RESULTS AND DISCUSSION

The extent of AFB_1toxin was highest from IIMR, New Delhi (Zone II) collected sample (161.0537 ppb), followed by farmers' seed sample from Saharanpur (153.5081 ppb) and farmers' seed sample from Bilaspur (144.7385 ppb) of same Zone-I. Lowest AFB_1 was observed from Almora (Zone-I) collected seed sample (0.0294 ppb). However, AFB_1 toxin range was noticed in Zone-I (0.0294-153.5081 ppb), Zone-II (0.1761- 161.0537 ppb ppb) and Zone-III (3.8366-53.1256 ppb) collected seed samples (table-1, fig.-1and plate-1). The conditions like high rainfall along with comparatively higher temperature during

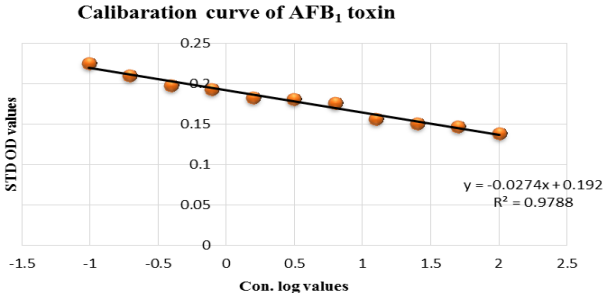

Fig.1. *Standard curve of different concentrations of AFB_1 toxin.*

cropping season also effect the activity of other microbes which might lead to AFB_1 toxin magnitude. Consequently, these conditions affect the general plant growth and seed health during and after the harvesting of crop (Abbas *et al.,* 2006; Kumar *et al.,* 2015). Aflatoxin production by the *Aspergillus* spp. is triggered by drought and high temperature during grain fill. Nitrogen deficiency, excessive plant population, poor root development and insect damage of grains may also induce Aflatoxin production in the field. When the weather conditions are favorable for the development of fungi, the fungus may produce Aflatoxins at any stages of production and transformations (Alptekin *et al.,* 2009).

Ono *et al.* (2001) investigated fumonisins and Aflatoxins in freshly harvested corn samples, using ELISA techniques. Fumonisins were detected in 147 (98 %) samples at a concentration range of 0.096 to 22.6 µg/g, while aflatoxins were detected in 17 (11.3 %). All the Aflatoxin positive samples (range 38.0-460.0 ng/g) came from Central Western region of Brazil and were co-contaminated with fumonisins. Alptekin *et al.,* (2009) observed that that the incidence of *Penicillium* spp. was significantly higher than *Fusarium* and *Aspergillus* from the corn samples at Turkey. However, 43 % of the samples were contaminated with AFB1 and themold counts ranged from 1 x 10^5-1 x 10^7cfu/g and aflatoxin levels ranged from 14.03-116.72 ppb.

Table 1. Screening of AFB_1 toxin levels in maize fresh grain sample collected from three maize growing zones.

S. No.	SD Con. (ppb)	Log	SDOD	SMOD-y	Log	Aflatoxin (in ppb)-x	Locations
1	100	2	0.1390	0.2340	-1.53102	0.0294	Almora
2	50	1.69897	0.1473	0.1939	-0.07079	0.8496	Kullu
3	25	1.39794	0.1507	0.1328	2.160584	144.7385	Bilaspur
4	12.5	1.09691	0.1570	0.2254	-1.21898	0.0604	Dhaulakuan
5	6.25	0.79588	0.1770	0.1975	-0.19894	0.6325	Kangra
6	3.125	0.49485	0.1810	0.1321	2.186131	153.5081	Saharanpur
7	1.5625	0.19382	0.1830	0.1843	0.280511	1.9077	Karnal
8	0.78125	-0.10721	0.1933	0.2127	-0.75414	0.1761	Ludhiana
9	0.390625	-0.40824	0.1987	0.1385	1.952555	89.6509	Pantnagar
10	0.195313	-0.70927	0.2107	0.1315	2.206971	161.0537	New Delhi
11	0.097656	-1.0103	0.2253	0.1454	1.699908	50.1081	Begusarai
12				0.1480	1.604136	40.1917	Varanasi
13				0.1447	1.725304	53.1256	Sabaur 1
14				0.1760	0.583942	3.8366	Sabaur 2

Note: SD: Standard, SDOD: Standard optical density, SMOD= Sample optical density

Saleemi, *et al.* (2012) identified toxigenic myco-flora and found that *Aspergillus parasiticus* produced higher concentrations of AFB_1 (Max.- 1374.23 ng g^{-1}) than *A. flavus* (Max.- 635.50 ng g^{-1}) from 82 maize grain and 8 maize-gluten meal samples. The present study also follows that the ELISA method is very convenient and most sensitive among many available methods. This method makes it possible to carry out the fast analysis of a considerable number of samples without any laborious stage of sample preparation.

Conclusion

Among the groups of toxins, AFB_1 was found to be one of the most potent environmental carcinogens as a class 1A. From above study, we have found that high level of Aflatoxin in maize indicated consumers risk for exposure to high levels of Aflatoxin. Concentration range was noticed in Zone-I (0.0294- 153.5081 ppb), Zone-II (0.1761- 161.0537 ppb ppb) and Zone-III (3.8366- 53.1256 ppb) collected seed samples. High levels of Aflatoxin in maize grain samples emphasized the need for regular surveillance and improved control of Aflatoxin levels. ELISA technique could be applied to the monitoring of Aflatoxin contamination in a vast number of samples in a cost and time effective manner.

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REFERENCES

Abbas, A. K., Zblotowicz, R. M., Weaver, M. A., Horn, B. W., Xie, W. and Shier, W. T. (2004). Comparision of cultural and analytical methods for determination of Aflatoxin produced by Mississippi Delta *Aspergillus* isolates. *Can. J. Microbio.,* 50: 193–199

Abbas, H. K., Cartwright, R. D., Xie, W. and Shier, W. T. (2006). Aflatoxin and fumonisin contamination of corn (maize, Zea mays) hybrids in Arkansas. *Crop Prot.,* 25:1-9

Alptekin Yasar, Duman, Ahmet Dogan, Akkaya and Murat Reis (2009). Identification of fungal genus and detection of Aflatoxin level in second crop corn grain. *Journal of Animal and Veterinary Advances,* 8(9):1777–1779

Ayejuyo, O. O., Olowu, R. A., Agbaje, T. O., Atamenwan, M. and Osundiya, M. O. (2011). Enzyme - Linked immunosorbent assay (ELISA) of Aflatoxin B_1 in groundnut and Cereal Grains in Lagos, Nigeria. *Research Journal of Chemical Sciences,* 1(8): 1–5

Chun, H. S., Kim, H. J., Ok, H. E., Hwang, J. and Chung, D. (2007). Determination of Aflatoxin levels in nuts and their products consumed in South Korea, *Food Chemistry,* 102: 385–391

Kumar, S., Sinha, A., Hooda, K. S. and Singh, V. (2015). Advanced detection techniques of toxigenic fungi and their management strategies. In: Sixth IJAA-JSPS, International conference on Contemporary Advances of Science and Technology, 7-9 Aug., p.145

Moss, M. O. (1998). Recent studies of mycotoxins. *J. Appl. Microbiol.* Symposium Suppl. 84: 62S–76S

Ono, E.Y.S., Ono, M.A., Funo, F.Y., Medina, A.E., Oliveira, T.C.R.M., Kawamura, O., Ueno, Y. and Hirooka, E.Y. (2001). Evaluation of fumonisin Aflatoxin co-occurrence in Brazilian corn hybrids by ELISA. *Food Additives and Contaminants,* 18(8):719–729

Oplatowska-Stachowiak, M., Sajic, N., Xu Y., Haughey, S. A., Mooney, M. H., Gong Y. Y., Verheijen, R. and Elliott, C.T. (2016). Fast and sensitive Aflatoxin B1 and total Aflatoxins ELISAs for analysis of peanuts, maize and feed ingredients. *Food Control,* 63:239–245

Rothschild, L. J. (1992). IARC classes AFB1 as class 1 human carcinogen. *Food Chem.,* 34: 62–66

Saleemi, Muhammad Kashif, Khan, Muhammad Zargham Khan, Ahrar (2012). Occurrence of toxigenic fungi in maize and maize-gluten meal from Pakistan. *Phytopathologia Mediterranea,* 51(1): 219–224