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Comparison of Aflatoxin Yield from Peanut Isolate of *Aspergillus flavus* with Other Isolates

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ABSTRACT

Background: Mouldy growth in food commodities by aflatoxin producing fungi is a serious health concern to animals as well as human beings due to the harmful effects of aflatoxins like hepatotoxicity, carcinogenicity, impairment of protein synthesis, formation of DNA adducts, *etc*. even in minute concentrations. A tropical climate with moderate temperature, high humidity and heavy rainfall provides optimal conditions for the growth of aflatoxin producing fungi like *Aspergillus flavus* in different food items. The current study was to examine if the substrate(s) in a food item has any role to play in aflatoxin production by *A. flavus.*

Methods: The fungus was isolated from ten different edible materials and identified using standard procedure. The isolates were cultured in potato dextrose agar slants keeping the culture conditions identical in triplicates. The toxin extracted using chloroform as solvent and downstream processing to get crude toxin was quantified on the basis of dry weight. Thin layer chromatography was done and the fluorescence of the toxins from different isolates was visualised under high wavelength of ultra-violet light with standard Aflatoxin B_1 chromatographed vis-a-vis to confirm presence of the toxin in all the extracted toxins.

Result: Results of the extracted toxin dry weight comparison between different isolates as well as the fluorescence intensity in the chromatograms revealed that contaminated peanut isolate of *A. flavus* yielded the highest amount of crude toxin. Thus, the results indicate that there are some factor(s) in peanut that promote(s) aflatoxin production by *A. flavus*. This information was further used for formulating potato dextrose agar media for isolation of aflatoxin producing fungi by incorporating peanut extract in the media and comparing the amount of crude toxin yield.

Key words: Aflatoxin production promoter, Aflatoxin, Peanut.

INTRODUCTION

Aflatoxicosis outbreak was first seen in 1960 in England better known then as the Turkey X disease and was subsequently identified by Thin Layer Chromatography (Rustom, 1997). Aflatoxicosis is caused by the fungus *Aspergillus flavus* and *Aspergillus parasiticus* which produce a group of low molecular weight toxic metabolites which are called aflatoxins. The four major aflatoxins, *i.e*., B_1, B_2 (Blue colour), G_1 and G_2 (Green colour) were named based on their fluorescence under UV light and retention factor in thin layer chromatography. Aflatoxin B_1 being a strong carcinogen, has been widely researched in detail and its ramifications on human and animal health is well known (Detroy *et al*., 1971; Wogan, 1975). Aflatoxin B_1 is heat stable and therefore it can still be present in the end product after processing (Moon *et al*., 2012). Aflatoxins result in severe liver intoxication causing haemorrhagic necrosis, proliferation of the bile duct and edematous condition (Wild, *et al*., 2015). 4.5 billion people from developing countries were reported to have chronic exposure to high amounts of aflatoxins and such exposure over a long duration, even at low concentrations would lead to increased risk of hepatocellular carcinoma and extrahepatic tumours (Gnonlonfin *et al.*, 2013). Aflatoxins are readily absorbed through the gastrointestinal route and respiratory route into blood circulation. They are also highly liposoluble compounds (Larsson and Tjalve, 2000; Agag, 2004). Being liposoluble, they are also deposited in the fatty tissues of livestock whose meat are consumed by humans and indirectly affect humans when they consume such meat. They are also deposited in eggs as well (Bintvihok *et al.*, 2002).

A tropical climate with heavy rainfall, moderate temperature range and high humidity produce optimum conditions for fungal growth. Therefore, chances of aflatoxin contamination due to mould growth in various food items are high. The aim of this study was to isolate *A. flavus* from different food sources kept in storage for a prolonged duration and to harvest aflatoxins from them and quantify the crude extract by dry weight and compare the toxin production. This was to ascertain if substrates in different food items played any role in toxin production. The results

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revealed that toxin production on peanut is highest compared to other isolates under same conditions of growth on media and toxin harvesting methods.

MATERIALS AND METHODS

Chemicals used were Chloroform (Fisher Scientific, U.S.A.), Acetone (Himedia, India), Lactophenol Cotton Blue (Himedia, India), Potato Dextrose Agar (Himedia, India) and Potato Dextrose Broth (Himedia, India). Glassware used were of Borosil, India. Microfuge tubes were of Eppendorf, Germany. The aflatoxin B_1 standard was from Himedia, India. Drying of extracts was done in Hot Air Oven (N.S.W.-142, India). Silica gel plates were from Himedia. UV fluorescence was observed under UV transilluminator and Gel Documentation system (Alpha Imager, U.S.A.).

Isolation and identification of fungal growth from different sources

Fungal isolates were collected from the following contaminated food items: coffee, round dry lemon, lemon, poultry feed, tea leaf, potato, silverberry, peanut, orange and banana and then inoculated on sterile Potato Dextrose Agar (PDA) in Petri dishes and incubated for five days at room temperature. The fungal isolates' growth on the Petri dishes was monitored daily. Lactophenol cotton blue staining was done as per Dutta, (1998) on fifth day post inoculation for identification of phialide structure.

Harvesting of toxin and estimation of crude aflatoxin content by dry weight

The fungal isolates were cultured on in triplicates in Potato Dextrose Agar test tube slants and incubated for 10 days at room temperature to obtain maximum aflatoxin production. Thereafter, 1 ml chloroform was added to the tube to extract the aflatoxins. Two such extractions were done and the extracts of one test tube pooled together in 2 ml centrifuge tubes. The crude extract in chloroform was dried overnight in hot air oven (NSW-142) at 60°C. The weights of the tubes before adding the extract and after drying were noted to obtain the dry weight of the crude toxin extract.

Thin layer chromatography and fluorescence under UV light

Thin layer chromatography (TLC) was done as per Dutta, (1998) with some modifications. The dried extracts in the tubes were reconstituted in 10 ìl to concentrate the aflatoxin content for thin layer chromatography. 10 µl was loaded on silica gel plates and TLC was run. Solvent system used was Chloroform: Acetone (9:1). TLC was run for 15 minutes. The fluorescence of the toxins under UV light was compared with 3 ìl of AFB_1 standard (Himedia) visually.

RESULTS AND DISCUSSION

Macroscopic colony characteristics of the isolated fungal isolates *i.e*., greenish white cotton like growth within 3 days with yellowish pigmentation on reverse view of the PDA Petri Dish were typical of *A. flavus*. *A. flavus* was microscopically confirmed by phialide structure by lactophenol cotton Blue staining as per Dutta, (1998).

Table 1: Mean±SD aflatoxin yield of different fungal isolates.

Source	Mean yield±SD (µg)
Coffee	0.42±0.03
Round dry lemon	0.49±0.02
Lemon	0.35±0.03
Poultry feed	0.34±0.02
Tea leaf	0.40±0.01
Banana peel	0.35±0.02
Potato	0.35±0.01
Silverberry	0.91±0.01
Peanut	1.02±0.04
Orange peel	0.93±0.02

The highest content of crude aflatoxin was found to be in peanut, silverberry and orange peel as observed in Table 1. This should be duly noted as these items are consumed by humans as well and mismanagement in storage conditions due to poor handling and improper storage facilities would ultimately lead to aflatoxin contamination.

Thin Layer Chromatography further supported the data in Table 1 as the deep bluish fluorescence intensity under high wavelength (380 nm) of ultraviolet light was high in case of peanut, silverberry and orange peel which as mentioned earlier had high dry weight of crude aflatoxin. Retention factors were in the range of 0.5 to 0.7 for all the extracts.

The results (Table 1) show that highest toxin production is in peanuts. In a study in Kenya, it was observed that contaminated peanuts harvested in humid agro-ecological zones had a high level of toxins (Mutegi *et al.*, 2009). In another report where a potent *A. flavus* Link isolate was inoculated in different food items, highest total aflatoxin was found to be on peanut (Wildman *et al.*, 1967). Mizoram also has high humidity which could promote toxin production in peanuts. Therefore, we can infer that there are some factor(s) or substrate(s) in peanut that promote toxin production by *A. flavus* because the growth and harvesting methods were same for all isolates. These factor(s)/ substrate(s) may stimulate expression of some genes essential for toxin production. If these factor(s)/ substrate(s) are also present in other food items, attempts can be made to inactivate it to reduce aflatoxin production which require further research.

CONCLUSION

A tropical humid climate with moderate temperature and heavy rainfall provides favourable conditions of growth of aflatoxigenic fungus most notably *A. flavus*. Aflatoxin contamination in different food items or commodities is very much likely as damp, humid conditions promote fungal growth as well as the food commodities being good substrates for their growth as well. Aflatoxin contamination is a serious health hazard to livestock and indirectly to

humans as well. The pathogenic effects of aflatoxins even at low concentrations are well known. Therapeutic measures are not very effective so prevention is of utmost importance. The spores of the fungus are very light and airborne and mould growth can occur in any area with optimum conditions. Storage conditions should be dry and there should not be water stagnation in the storage facility to avoid aflatoxin contamination and rain water should not be allowed to drip into the feed storage area. Fungal contaminated items should be discarded properly to avoid spread of the spores of the fungus which might contaminate nearby sources. Through optimum and hygienic storage conditions as well as optimum management of the housing and rearing system for livestock, the harmful effects of aflatoxins can be mitigated to a manageable level.

The factor(s) contributing to increased toxin production in peanut could be a valuable asset to counteract toxin contamination. Further studies are required to identify this factor(s) and how it acts in the toxin production mechanism whether by gene expression or some other pathway. The presence of the factor(s) in other food sources has to be studied. If it is common in different food sources then it could prove helpful to reduce toxin production across different food commodities.

Conflict of interest: None.

REFERENCES

Agag, B.I. (2004). Mycotoxins in foods and feeds: Aflatoxins. Assiut University Bulletin for Environmental Researches. 7(1): 173-191.

Bintvihok, A., Thiengnin, S., Doi, K., and Kumagai, S. (2002). Residues of aflatoxins in the liver, muscle and eggs of domestic fowls. Journal of Veterinary Medical Science. 64: 1037-1039.

Detroy, R.W., Lillehoj, E.B., and Ciegler, A. (1971). Aflatoxin and Related Compounds. In: Microbial toxins, [Ciegler, A., Kadis, S. and Ajl, S.J. (ed.)] Academic Press Inc., New York. vol. 6: 4.

Dutta, T.K. (1998). Comparison of enzyme-linked immunosorbent assay with thin layer chromatography for aflatoxin B_1 determination in naturally contaminated feeds. M.V.Sc. Thesis, submitted to I.V.R.I Deemed University, Izatnagar.

Gnonlonfin, G.J. B., Hell, K., Adjovi, Y., Fandohan, P., Koudande, D.O., Mensah, G.A., *et al.* (2013). A review on aflatoxin contamination and its implications in the developing world: A Sub-Saharan African perspective. Critical Reviews in Food Science and Nutrition. 53: 349-365.

Larsson, P. and Tjalve, H. (2000). Intranasal instillation of Aflatoxin B1 in rats: Bioactivation in the nasal mucosa and neuronal transport to the olfactory bulb. Toxicological Sciences. 55: 383-391.

Moon, J., Kim, G., and Lee, S. (2012). A gold nanoparticle and aflatoxin B1-BSA conjugates based lateral flow assay method for the analysis of aflatoxin B1. Materials. 5(4): 634-643.

Mutegi, C.K., Ngugi, H.K., Hendriks, S.L., and Jones, R.B. (2009). Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya. International Journal of Food Microbiology. 130(1): 27-34.

Rustom, I.Y.S. (1997). Aflatoxin in food and feed. Occurrence, legislation and inactivation by physical methods. Food Chemistry. 59: 57-67.

Wild, C.P., Miller, J.D., and Groopman, J.D. (2015). Mycotoxin control in low-and middleincome countries. France: International Agency for Research on Cancer Lyon.

Wildman, J. D., Stoloff, L., and Jacobs, R. (1967). Aflatoxin production by a potent Aspergillus flavus Link isolate. Biotechnology and Bioengineering. 9(3): 429-437.

Wogan, G. (1975). Mycotoxins. Annual Reviews in Pharmacology. 15: 437-451.