



HPLC Analysis of Aflatoxin B₁ Contamination of Local and Foreign Brands of Cigarette Tobacco Popularly Consumed in Niger State North-Central Nigeria

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Abstract

Under favorable growth conditions, fungi produced aflatoxins. Aflatoxin B₁ (AFB₁) categorized as a carcinogen is the most toxic and subject of regulation in foods and feeds in many countries. AFB₁ contamination has been reported in a wide range of products including cigarette tobacco due to improper processing and storage conditions. This research aim at determining the concentration load of AFB₁ mycotoxin in brands of cigarette tobacco commonly consumed in Lapai and Minna metropolis of Niger State Nigeria. Thirty-six (36) brands of cigarette tobacco were purchased in open markets in Minna and Lapai towns of Niger state, Nigeria. Residual AFB₁ was extracted from the sample using standard procedures. HPLC analysis was used for the mycotoxin quantification. The result showed that all cigarette samples produces AFB₁ with a concentration of 302 µg/mL and 18 µg/mL as the highest and lowest AFB₁ concentration respectively for the local – Nigeria brands compared to 166 µg/mL and 8 µg/mL AFB₁ concentration observed for the foreign brands. The findings from this study provide sufficient levels of contamination to pose significant health hazard for the consumer of the tobacco. Careful processing and storage is therefore advocated to eliminate or reduce the mycotoxin contamination of the cigarette brands.

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Keywords and phrases: Aflatoxin B₁, cigarette tobacco, HPLC, mycotoxin.

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Introduction

Mycotoxins are secondary metabolites produced by fungi that frequently contaminate agricultural commodities. Aflatoxins, produced by common mold fungi in the genus *Aspergillus*, are one of the most important groups of mycotoxins. Among the most commonly occurring aflatoxins are aflatoxin B₁, G₁, B₂, G₂) (Figure 1). Aflatoxin B₁ (AFB₁) is the most toxic and has been the subject of regulation in foods and feeds in many countries, including the United States [1]. AFB₁ contamination can occur in a varied range of products due to improper storage conditions and has been shown to be hepatotoxic, carcinogenic, and teratogenic, as well as causing other less specific symptoms such as weight loss and impaired immune systems [2]. As a result of its harmful nature, AFB₁ has been included as a carcinogen on the list of Harmful and Potentially Harmful Constituents (HPHCs) in tobacco products as regulated by the Food and Drug Administration (FDA) [3].

Tobacco is one among the 25% of world crops affected by mycotoxins every year [4]. Diverse microorganisms community ranging from bacteria (both Gram positive and Gram negative), bacterial spores and toxins (endotoxins and exotoxins), molds, yeasts, as well as fungal spores and their secondary metabolites such as aflatoxin B₁ are involved in the production process of cigarette from tobacco, with the spores of such fungi capable of surviving the entire manufacturing process and the level of these toxins production influenced by the substrate on which the fungi grow as well as the moisture level, temperature, and presence of competitive micro-flora [5,6].

Aflatoxin contamination of tobacco and tobacco related products have long been reported. *Aspergillus flavus* growth on flue-cured tobacco have been observed [7] with several aspergilli reported in the stored leaves of tobacco [8]. AFB₁ has been reported in tobacco products such as cigarettes [9] and chewable tobacco mixtures including other plant material such as betel leaf [10]. Exposure to aflatoxins in cigarettes may be mitigated by their potential destruction in the combustion process [11]. AFB₁ has traditionally been detected in commodities by use of HPLC with fluorescence detection [12] but with HPLC-MS/MS and UHPLC-MS/MS methods becoming more common [13-15]. In this study, we screened both local and foreign brands of tobacco cigarettes consumed in Lapai and Minna towns of Niger State Nigeria to ascertain the extent of aflatoxin B₁ contamination using HPLC method.

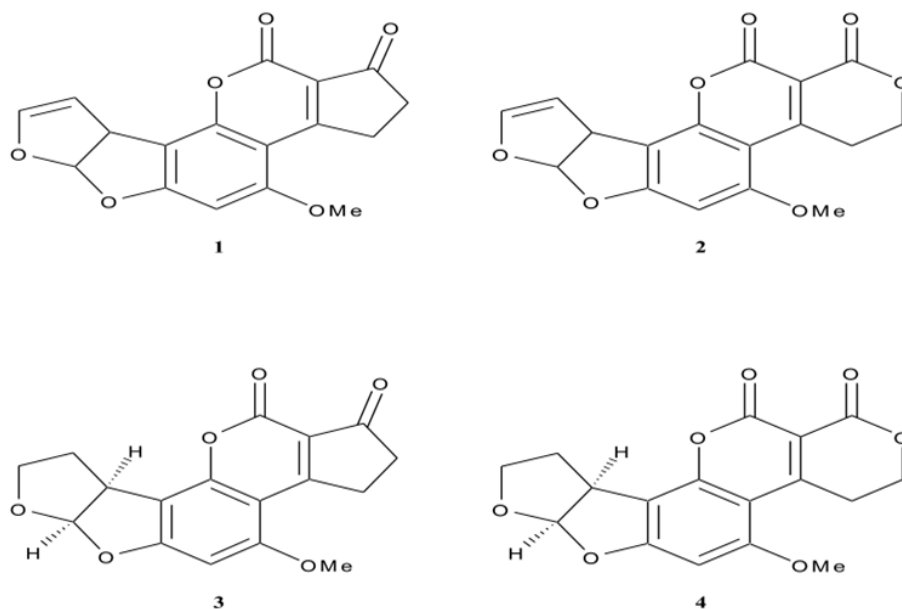


Figure 1. Chemical structures of aflatoxins: aflatoxin B₁ (AFB₁) (1), aflatoxin G₁ (AFG₁) (2), aflatoxin B₂ (AFB₂) (3), aflatoxin G₂ (AFG₂) (4).

Materials and Methods

Sample collection

Thirty six (36) different cigarette brands were procured from randomized cigarette wholesale and retail outlets in Lapai and Minna towns of Niger State.

Extraction of residual AFB₁

Twenty five (25g) of the tobacco in each of the cigarettes was weighed into a 250 mL Erlenmeyer flask and extracted for 30 minutes on a wrist action shaker using 125 mL of methanol – water mixture (55:45 v/v), 50 mL of n-hexane and 1g of sodium chloride. The mixture was filtered through Whatmann filter paper and the residue discarded. The filtrate was allowed to settle, and the lower methanol phase was collected into a separating funnel. It was then extracted 3 times with 25ml chloroform. The chloroform extracts were concentrated into the vials and evaporated to dryness. The dry film was dissolved with 400µl of the mobile phase (water: methanol: acetonitrile 60:20:20) [16].

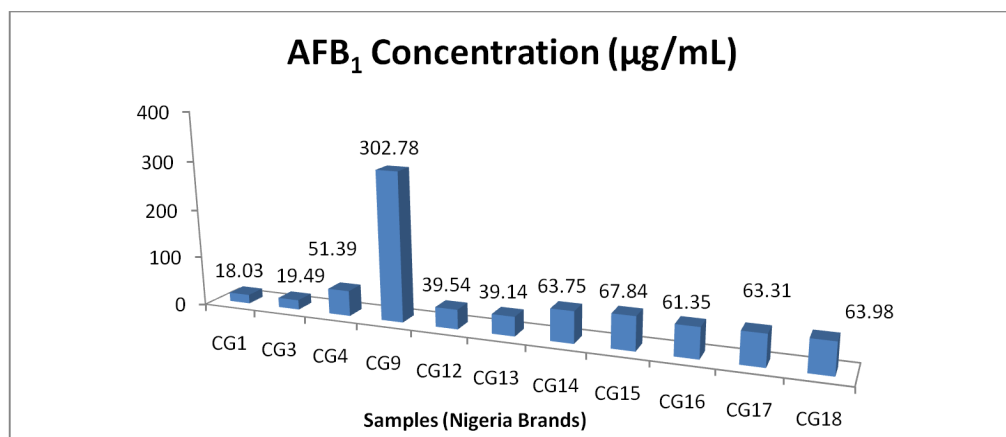
Quantification of AFB₁ using HPLC analysis

AFB₁ was quantified by reversed-phase HPLC methods. Aflatoxin B₁ was analysed

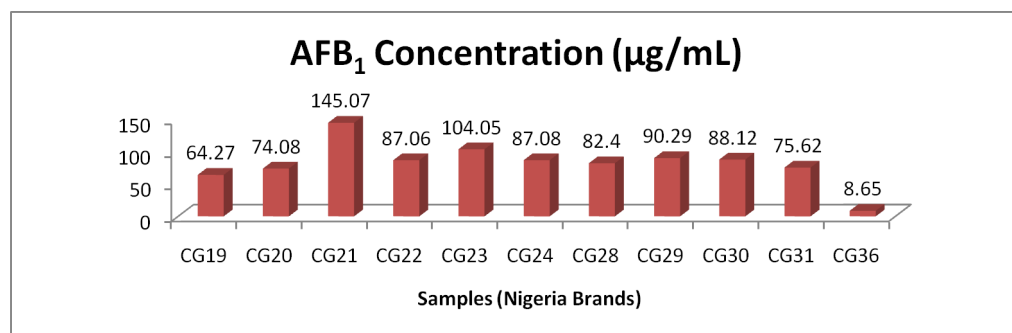
on a Buck Instruments high performance liquid chromatography (HPLC) with UV-detection wavelength of 365nm. A C18 column type, 250 x 4.6mm with 5 micron particle size was used at an ambient temperature of 25°C. The mobile phase was water:methanol:acetonitrile in the ratio of 60:20:20 at a flow rate of 1ml/min. the injection volume was 20µl.

Results

AFB₁ quantification of the cigarette samples using HPLC analysis reveals varying concentrations of AFB₁ in both local and foreign cigarette brands as represented in Figures 2 and 3.



(a)



(b)

Figure 2. (a) and (b): Aflatoxin B₁ concentration of analyzed local (Nigerian) brands of cigarette tobacco.

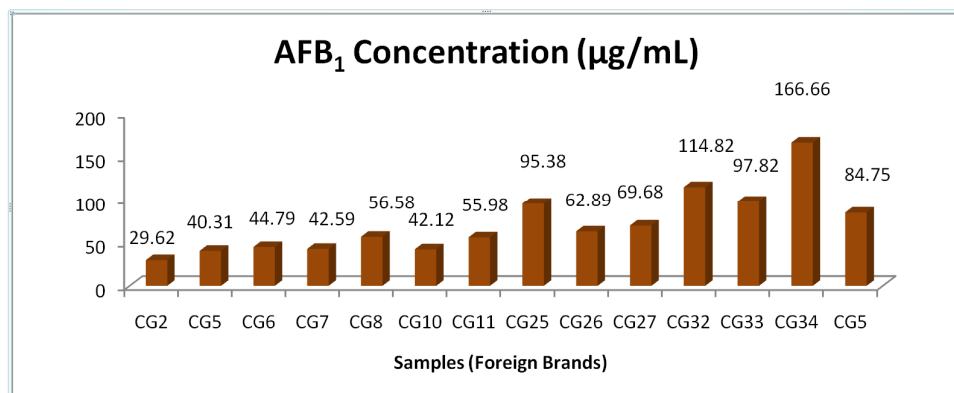


Figure 3. Aflatoxin B₁ concentration of analyzed foreign brands of cigarette tobacco.

Key: CG 2, 26, 27, 32, 33 (South Korea); CG 5, 34, 35 (Senegal); CG 6, 7, 8, 11 (UAE); CG 10 (Germany); CG 25 (Britain).

Discussion

Although fungi may be present without producing any toxin, in an earlier report by Ndagi *et al.* [17], different colonies of fungi ranging from *Apergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Chrysonillia sitophilia* and *Aspergillus flavus* were all isolated from Nigeria and foreign brands of cigarette tobacco [17]. This finding was confirmed by Pauly *et al.* [18] who isolated diverse microbes and microbial toxins from more than 90% individual tobacco flakes from eight different popular US cigarette brands. They reported that these toxins are released from the cigarette during smoking, and carried into mainstream smoke that is sucked deep into the lung. Several other researchers have reported that tobacco cigarettes harbor a plethora of bacteria (Gram-positive and Gram-negative), fungi (mold, yeast), spores, and is rich in endotoxin [19-22]. This is expected since microorganisms are involved in the production process of tobacco cigarette as well as mycotoxin producing fungi earlier isolated from these cigarette brands. It becomes imperative therefore to ascertain the concentration of aflatoxin B₁ mycotoxin concentration in the brands of cigarettes. The results of the mycotoxin concentration show that the local brands (302 µg/mL and 18 µg/mL as the highest and lowest aflatoxin concentration respectively) compared to their foreign counterpart (166 µg/mL and 8 µg/mL as the highest and lowest aflatoxin concentration respectively). This could however be attributed to better processing of the foreign brands than the local Nigeria brands since processing methods had been reported to reduce fungi contamination and consequently their mycotoxins production [17].

Conclusion

The HPLC analysis of the tobacco cigarette brands showed that all the cigarette brands (Nigeria and Foreign) were not only contaminated with fungi but produce AFB₁ with varying concentrations. The presence of fungi and its associated mycotoxins confirms that cigarettes pose a significant health risk to its consumers although the claim of a 100% aflatoxin B₁ transfer to smoke during tobacco combustion had been refuted by several research and factory documents.

References

- [1] USDHHS, Chemical Contaminants, Metals, Natural Toxins & Pesticides, Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed, U.S. Food and Drug Administration, 2012a. <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/CheicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm> [Accessed: 5th November, 2020]
- [2] CAST, Mycotoxins: Risks in Plant, Animal and Human Systems Task Force Report No 139, Council for Agricultural Science and Technology, Ames, IA, USA, 2003, pp. 23.
- [3] USDHHS, Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke; Established List. U.S. Food and Drug Administration, 2012b. <http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297981.pdf> [Accessed: 5th November 2020].
- [4] Food and Agricultural Organization of the United Nations Food and Nutrition Paper 64, Worldwide regulations for mycotoxins, A compendium, Food and Agricultural Organization, Rome, Italy, 1997.
- [5] D.A. Musa and T.A. Gbodi, *Mycotoxin Associated Risks of Cigarette Tobacco Sold in Nigeria*, Lambert Academic Publishing, Beau Bassin 71504, Mauritius, 2020, pp. 31-35.
- [6] J.J. Verweij, A. Kerremans, P. Voss and J.F.G.M. Meis, Fungal contamination of tobacco and marijuana, *Journal of the American Medical Association* 284(22) (2012), 2875-2881. <https://doi.org/10.1001/jama.284.22.2875>
- [7] H.E. Pattee, Production of aflatoxins by *Aspergillus flavus* cultured on flue-cured tobacco, *Applied Microbiology* 18 (1969), 952-953. [PubMed: 16349871] <https://doi.org/10.1128/AM.18.5.952-953.1969>
- [8] S.K. Varma, R.A. Verma and A.K. Jha, Ecotoxicological aspects of *Aspergilli* present in the phylloplane of stored leaves of chewing tobacco (*Nicotiana tabacum*), *Mycopathologia* 113 (1991), 19-23. [PubMed: 2014047] <https://doi.org/10.1007/BF00436380>

- [9] L.E. Edinboro and H.T. Karnes, Determination of aflatoxin B₁ in side stream cigarette smoke by immunoaffinity column extraction coupled with liquid chromatography/mass spectrometry, *Journal of Chromatography* 1083 (2005), 127-132. [PubMed: 16078698] <https://doi.org/10.1016/j.chroma.2005.06.032>
- [10] R.J. Verma, A.S. Kolhe and H.C. Dube, Aflatoxin contamination in chewing products, *Proceedings of the National Academy of Sciences India, Sect B.* 65 (1995), 167-170.
- [11] T.C. Tso and T. Sorokin, Examination of aflatoxin B₁ in leaf tobacco and in cigarette smoke condensate, *Beiträge zur Tabakforschung International* 4 (1967), 18-20. <https://doi.org/10.2478/cttr-2013-0162>
- [12] M.W. Trucksess, C.M. Weaver, C.J. Oles, F.S. Jr. Fry and G.O. Noonan, Determination of aflatoxins B₁, B₂, G₁, and G₂ and ochratoxin A in ginseng and ginger by multi toxin immunoaffinity column cleanup and liquid chromatographic quantitation: collaborative study, *Journal of AOAC International* 91 (2008), 511-523. [PubMed: 18567295] <https://doi.org/10.1093/jaoac/91.3.511>
- [13] B. Huang, Z. Han, Z. Cai, Y. Wu and Y. Ren, Simultaneous determination of aflatoxins B₁, B₂, G₁, G₂, M₁, and M₂ in peanuts and their derivative products by ultra-high-performance liquid chromatography-tandem mass spectrometry, *Analytica Chimica Acta* 662 (2010), 62-68. [PubMed: 20152266] <https://doi.org/10.1016/j.aca.2010.01.002>
- [14] W. Li, T.J. Herrman and S.Y. Dai, Determination of aflatoxins in animal feeds by liquid chromatography/tandem mass spectrometry with isotope dilution, *Rapid Commun Mass Spectrometry* 25 (2011), 1222-1230. [PubMed: 21491528] <https://doi.org/10.1002/rcm.4979>
- [15] C. Cervino, S. Asam, D. Knopp, M. Rychlik, M and R. Niessner, Use of isotope-labeled aflatoxins for LC-MS/MS stable isotope dilution analysis of foods, *Journal of Agriculture and Food Chemistry* 56 (2008), 1873-1879. [PubMed: 18303822] <https://doi.org/10.1021/jf073231z>
- [16] A.O. Elzupir, Y.M.H. Younis, M.H. Fadul and A.M. Elhussein, Determination of aflatoxins in animal feed in Khartoum State, Sudan, *Journal of Animal and Veterinary Advancement* 8(5) (2009), 1000-1003. <https://doi.org/10.3923/javaa.2009.1000.1003>
- [17] M.S. Ndagi, D.A. Musa and C. Ogbiko, Investigation of fungal contamination of different cigarette tobacco brands consumed In Lapai and Minna metropolis of Niger State Nigeria, *Journal of Science Technology and Education* 8(3) (2020), 357-364.
- [18] J.L. Pauly, J.D. Waight and G.M. Paszkiewicz, Tobacco flakes on cigarette filters grow bacteria: a potential health risk to the smoker, *Immunology Research* 17(Suppl 1) (2007), <https://doi.org/10.1136/tc.2007.022772>

- [19] J. Papavassiliou, G. Piperakis and U. Marcelou-Kinti, Mycological flora of cigarettes, *Mycopathologia et Mycologia Applicata* 44 (1971), 117-120. <https://doi.org/10.1007/BF02051879>
- [20] R. Marjut and U. Jukka, Exposure to microbes, endotoxins and total dust in cigarette and cigar manufacturing: an evaluation of health hazards, *The Annals of Occupational Hygiene* 44(6) (2000), 467-473. [https://doi.org/10.1016/S0003-4878\(00\)00012-0](https://doi.org/10.1016/S0003-4878(00)00012-0)
- [21] O.M. El-Maghraby and M.A. Abdel-Sater, Mycoflora and natural occurrence of mycotoxins in tobacco from cigarettes in Egypt, *Zentralblatt für Mikrobiologie* 148(4) (1993), 253-264. [https://doi.org/10.1016/S0232-4393\(11\)80101-0](https://doi.org/10.1016/S0232-4393(11)80101-0)
- [22] J.L. Pauly, L.A. Smith and M.H. Rickert, Review: Is lung inflammation associated with microbes and microbial toxins in cigarette tobacco smoke?, *Immunology Research* 46 (2010), 127-136. <https://doi.org/10.1007/s12026-009-8117-6>