



Comparative Study on the Incidence of *Aspergillus flavus* in Farmer's Field and Stored Maize (*Zea mays*) Seed in Northern Region of Ghana

Elias Nortaa Kunedeb Sowley¹, Frederick Kankam^{1*} and Edmond Tawiah¹

¹Department of Agronomy, Faculty of Agriculture, University for Development Studies, P.O.Box 1882, Tamale, Ghana.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/APRJ/2018/v1i226269

Editor(s):

(1) Dr. Vineet Kaswan, Assistant Professor, Department of Biotechnology, College of Basic Science and Humanities, Sardarkrushinagar Dantiwada Agricultural University, Gujarat, India.

Reviewers:

(1) Okpo, O. Ngozi, Federal College of Agricultural Produce Technology, Nigeria.

(2) Isela Quintero Zapata, Universidad Autónoma de Nuevo León, Mexico.

(3) R. Mahalakshmi, India.

(4) Sherif Mohamed El-Kadi, Damietta University, Egypt.

Complete Peer review History: <http://www.sciencedomain.org/review-history/26789>

Original Research Article

Received 05 July 2018

Accepted 30 September 2018

Published 23 October 2018

ABSTRACT

Aspergillus flavus is predominant among other fungi species responsible for aflatoxin contamination of crops on the field and in storage. A study was conducted to determine the incidence of *Aspergillus flavus* in maize seeds from various sources. Sixty (60) maize seed samples were collected from farmers and marketers from different storage structures within Tamale metropolis, Savelugu and Tolon districts, northern region of Ghana. Three different fungi species were isolated from the maize samples. These were *Aspergillus flavus*, *Aspergillus niger* and *Fusarium* spp. Total fungi species differed significantly. Tamale metropolis recorded the least occurrence of fungi species. *Aspergillus flavus* recorded varied occurrences across all three districts with the highest incidence recorded in Savelugu (42%). *Aspergillus flavus* was found predominant in maize from the markets than maize from the farmers. The incidences of *Aspergillus flavus* varied with the method of storage. Storage using cocoa sack recorded the highest incidence (69%), with the lowest (13%) in hanging shed. *Aspergillus flavus* was however found to be associated with maize from all three districts studied. Proper drying and use of proper storage structures are recommended as a way of combating the high incidences of *Aspergillus flavus*.

*Corresponding author: E-mail: fkankam@uds.edu.gh;

Keywords: Disease incidence; *Aspergillus* spp.; *Fusarium* spp.; mycotoxins; storage structures; maize.

1. INTRODUCTION

Maize (*Zea mays*) is the most important staple crop in Africa [1]. It accounts for over 50% of the total cereal production in Ghana, and annual yield has been reported to be growing around 1.1% [2] with postharvest losses of between 5 and 70% [3]. In Ghana, maize is grown in almost all parts of the country and is the main source of carbohydrate for both rural and urban families [4]. Maize crop is produced mainly by the peasant farmers under rainfed conditions [5].

Aspergillus, *Penicillium* and *Fusarium* species are the tiny moulds that are mostly found in stored cereal grains and oilseeds. These fungi can cause food spoilage, bio-deterioration and are capable of producing different mycotoxins. Surprisingly, *Aspergillus* species have been identified to be the most toxin-producing species in various grains, legumes, oilseeds and foods as well as feeds [6,7,8]. Among all these fungal species, *Aspergillus flavus* is the predominant fungus responsible for aflatoxin contamination of crops before storage and in storage [9,10]. Aflatoxin causes the most concern among all the mycotoxins [11] because of its carcinogenic and immunosuppressing effects in both humans and domestic animals [12]. Improper drying, high relative humidity and temperature, farmers' production practices, early and delayed harvesting and poorly constructed storage structures and storage practices, as well as stress-induced crop growing period, are the factors that expose grains to *A. flavus* infection [13,14].

According to Strosnider et al. [15] and Williams et al. [16], aflatoxin is one of the most serious food safety related problems of the world, and the common cause of these aflatoxins is incidence of *Aspergillus flavus* [17]. Aflatoxin consumption leads to a disease called aflatoxicosis which occurs in two forms; acute aflatoxicosis which result in death and chronic aflatoxicosis which also results in cancers, immune suppressions and other slow pathological conditions [18]. Many control measures, such as chemical, biological, and physical means, have been employed to reduce or eliminate these aflatoxin-producing fungi in maize, but none have proven economically feasible [19]. The effect of aflatoxin-producing fungi associated with stored maize seed needs to be investigated. The purpose of this study, therefore, was to

determine the occurrence of *Aspergillus flavus* in maize seed from various sources within Tolon, Tamale and Savelugu districts of the Northern Region of Ghana.

2. MATERIALS AND METHODS

2.1 Study Area

The study site consisted of three districts within the Northern Region of Ghana namely Tamale Metropolis, Tolon and Savelugu. The Tamale metropolis is located at latitude 9°25' N and longitude 0°51' West and has an elevation of approximately 173 m (568 feet). Tolon district is located at latitude 9°25' North and longitude 0°59' West. Savelugu is located at latitude 9°37' North and longitude 0°50' West. The Districts are characterised by a single rainy season which starts in late April with little rainfall, rising to its peak in July-August and declining sharply and coming to a complete halt in October-November. Daily temperatures vary seasonally. During the rainy season, residents experience high humidity and heavy thunderstorms with slight sunshine, compared to the dry season which is characterised by dry harmattan winds from November to February and high sunshine from March to May. The mean annual rainfall ranges from 950 mm – 1200 mm. The main vegetation is grassland, interspersed with guinea savannah woodland, characterised by drought-resistant trees such as acacia (*Acacia longifolia*), mango (*Magnifera indica*), baobab (*Adansonia digitata*), shea (*Vitellaria paradoxa*), dawadawa (*Parkia biglobosa*), and neem (*Azadirachta indica*). Major tree species include the shea, dawadawa, and mango, which are economic trees and form an integral part of the livelihood of the natives.

2.2 Maize Sample Collection

Maize seed samples were collected from Tamale, Savelugu, and Tolon Districts. Twenty (20) samples were taken from each District. Ten (10) maize samples were randomly taken from ten different farmers within each District. Samples were collected from three (3) communities in each district. Ten (10) maize samples were taken from four different markets across each of the Districts using random sampling method. Maize samples were collected from farmers and vendors based on the used storage structures and duration in the field. The

samples were packed in paper bags and kept under dry conditions in the laboratory to prevent contact with moisture and contaminants.

2.3 Isolation and Identification of Fungi

2.3.1 Media preparation

The media was prepared according to the instructions of the manufacturer (Oxoid, UK). Thirty-nine grams (39 g) of Potato Dextrose Agar (PDA) was dissolved in one (1) litre of distilled water in a conical flask. The conical flask was covered with aluminium foil to prevent contamination, condensation and loss of moisture. The mixture was stirred and heated with the magnetic stirrer and the heating mantle respectively to obtain a homogenous mixture. The mixture was autoclaved at 121°C for 15 minutes and allowed to cool afterwards. About 20 ml of the prepared media was poured into each sterilised petri dish in a controlled environment (lamina flow hood) and allowed to solidify. The plates were now ready to be used.

2.3.2 Isolation

Fifteen seeds from each sample were surface sterilised in 3% sodium hypochlorite (NaOCl) solution for one minute to prevent the fungi from being killed while reducing surface contaminants at the same time. The maize seeds were rinsed in three changes of distilled water and left to dry on sterile filter papers. Five seeds were placed on PDA plates, and each replicated three (3) times. The plated maize seeds were incubated at a room temperature of 25°C for five days. After incubation, mixed cultures of various species of fungi were obtained from the plated maize. The mixed cultures were sub-cultured by transferring a loop full from each of the species on to PDA plates. The inoculated plates were incubated at 25°C for five days.

2.3.3 Identification of *Aspergillus flavus*

Aspergillus flavus was identified based on colony characteristics, strain morphology and microscopic features described by Klich [20].

2.3.4 Determination of Incidence of *Aspergillus flavus*

The incidences of *Aspergillus flavus* isolated from maize using Potato Dextrose Agar (PDA) were enumerated according to the method described by Cotty (1994). Other species of *Aspergillus* isolated were also identified and their incidences determined. This was done by

determining the percentage occurrence from the total counts of each *Aspergillus* species from each of the three districts.

2.4 Statistical Analyses

Data were subjected to Analysis of Variance (ANOVA) using generalised linear model (GLM) in Genstat (9th Edition), and the means were separated using the least significant difference (LSD) at 5% probability.

3. RESULTS

3.1 Fungal species in Maize Samples from the Three Districts

Three different species of fungi were isolated from all sixty samples collected. These were *Aspergillus flavus*, *A. niger* and *Fusarium* spp. Samples from Savelugu recorded the highest percentage occurrence of *A. flavus* (42%) while those from Tamale recorded the lowest (24%). Samples from Savelugu recorded the highest percentage occurrence of *A. niger* (50%) while Tamale and Tolon recorded the lowest (25%). For *Fusarium* spp., samples from both Tamale and Tolon recorded the highest (39%) percentage occurrence while the lowest (24%) was recorded in samples procured from Savelugu. In general, there were significant differences ($p < 0.05$) among all the districts about the percentage occurrence of the individual fungi species (Fig. 1).

3.2 Fungal species in Samples among Farmers Stored Seeds and Seeds from Markets

Generally, samples from the markets recorded the highest percentage occurrence of fungi as compared to those of the farmers. Among the market samples, *A. flavus* recorded the highest occurrence (41%) followed by *A. niger* (28%) and *Fusarium* spp. (31%). However, among the farmers' samples, *Fusarium* spp. recorded the highest occurrence (39%) while *A. flavus* and *A. niger* recorded 32% and 29%, respectively (Fig. 2). In general, there was no significant difference ($p > 0.05$) in the percentage occurrence of the individual fungi for their source.

3.3 Percentage Occurrence of *Aspergillus flavus* in Samples from the Markets and Farmers

Generally, samples from farmers and markets recorded the considerably high occurrence of *A.*

flavus. However, samples from the market recorded the highest percentage occurrence (62%) while the lowest (38%) was recorded in samples from farmers (Fig. 3). There was a highly significant difference ($p < 0.05$) among the farmers and markets concerning the occurrence of *A. flavus*.

3.4 Effect of Storage Method on the Occurrence of *A. flavus*

Among all the samples collected from the three districts, samples from cocoa sacks recorded the highest percentage occurrence of *A. flavus*

(69%) followed by fertiliser sack (18%) with hanging sheds as the least (13%) (Fig. 4). There were significant differences ($p < 0.05$) in the occurrence of *A. flavus* among the three methods of storage.

4. DISCUSSION

Generally, *Aspergillus flavus* was present in maize from all the three districts (Savelugu, Tamale, and Tolon). This affirms the report by Groopman and Donahue [21] that maize and its products are known to be prone to contamination by fungi that produce secondary metabolites

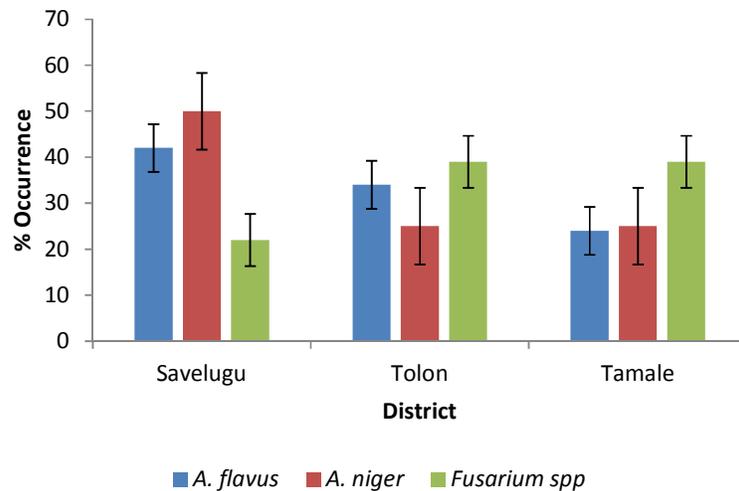


Fig. 1. Percentage occurrence of fungal species in maize samples from the three districts

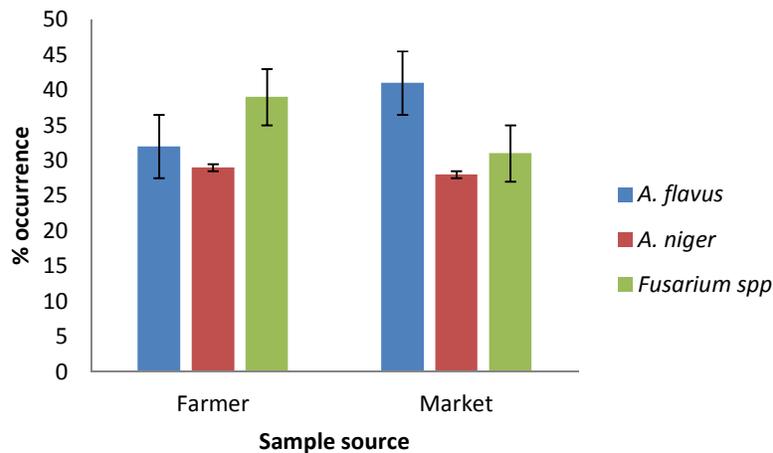


Fig. 2. Percentage occurrence of fungal species in maize samples from the two sources

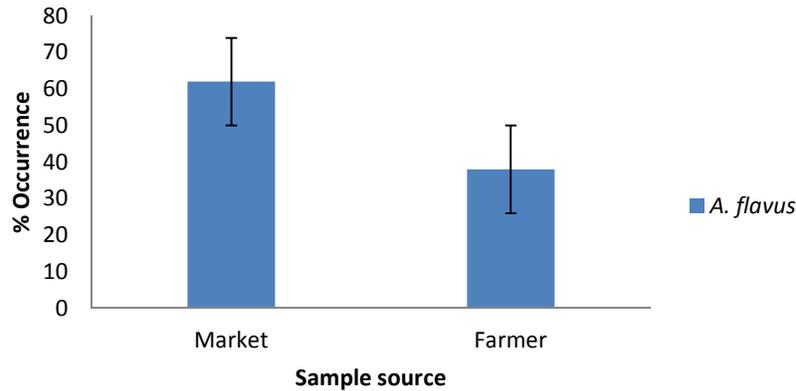


Fig. 3. Percentage occurrence of *A. flavus* in maize sample from the two sources

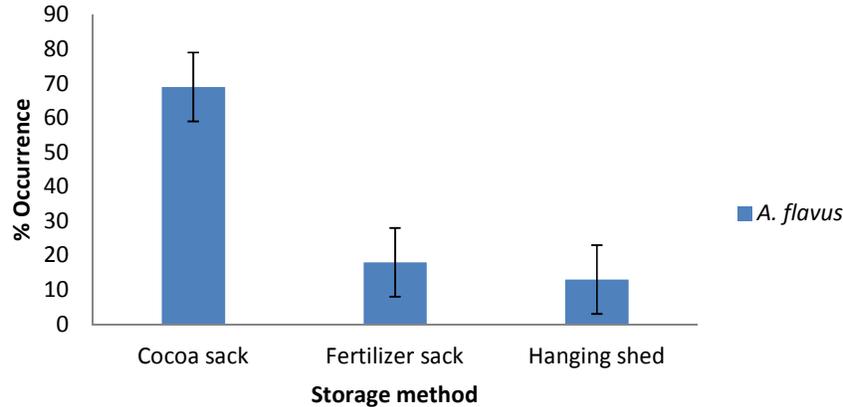


Fig. 4. Effect of maize storage methods on the occurrence of *A. flavus*

such as aflatoxin. It also agrees with the report by Atehnkeng et al. [22] and Gachara et al. [23] which indicated that *Aspergillus flavus* is the most predominant member of *Aspergillus* species in West African and the United States soils.

There were considerably high incidences of *Aspergillus flavus* in maize from all sources, and this could be mainly attributed to the high and wide range of temperatures in the study areas. This affirms the report by Northolt and van Egmond [24] that *Aspergillus* strains have been reported to survive in a wide range of temperatures from 19 to 35°C. It also confirms the report of Diener et al. [25] that optimal conditions for fungal development are 36 to 38°C with high humidity of above 85%.

Generally, high incidences of *A. flavus* were recorded in maize from the different cocoa sacks, fertiliser sacks, hanging sheds. It is likely that the storage conditions and the storage structures greatly influenced the mycoflora in storage. This could be attributed to the exposure of the maize to unfavourable conditions which favoured the development of the fungi. This agrees with the report by Fandohan et al. [26] that some storage structures do not safeguard maize from moisture pick-up, mould infection as well as protection of the grains against aflatoxin contamination. It also confirms the report by Hell et al. [27] and Udoh et al. [28] who indicated that many farmers nowadays store their grains in sacks, especially polypropylene which is not airtight, with evidence that this method facilitates fungal contamination and aflatoxin development.

5. CONCLUSION

Aspergillus flavus was associated with maize seeds collected from farmers and markets within Tamale metropolis, Savelugu and Tolon districts. However, its occurrence varied across the three districts studied. Very high incidence was recorded in maize seeds from Savelugu district whereas very low incidence was as well recorded in Tamale metropolis. Generally, maize collected from the market recorded higher incidences of the fungi than maize collected from farmers. Apart from *Aspergillus flavus*, *A. niger* and *Fusarium* spp. were also isolated from the maize samples. It is possible that all maize seeds which recorded high incidences of *Aspergillus flavus* were contaminated with high levels of aflatoxins because *A. flavus* is the major producer of aflatoxins which poses various threats to livelihood. It is therefore prudent to combat this fungus to its minimum.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alene AD, Menkir A, Ajala SO, Badu-Apraku B, Alanrewaju AS, Manyong VM, Ndiaye A. The economic and poverty impacts of maize research in West and Central Africa. *Agricultural Economics*. 2009;40:535-550.
2. Ragasa C, Chapoto A, Kolavalli S. Maize Productivity in Ghana. GSSP Policy Note 5. Washington, D.C. International Food Policy Research Institute (IFPRI); 2014. Available:http://ebrary.ifpri.org/cdm/ref/collection/p15738coll2/id/1282_63
3. FAO. (Food and Agricultural Organization) Statistical Division; 2012. Available:<http://faostat.fao.org>
4. Morris ML, Tripp R, Dankyi AA. Adoption and impacts. A case study of the of improved Ghana grains maize production development project technology: Adoption and Impacts of Improved Maize Production Technology: A Case Study of the Ghana Grains. Development; 2000.
5. SARI. (Savanna Agricultural Research Institute). SARI Annual Report; 1996.
6. Magnoli C, Chiacchiera SM, Miazzo R, Palacio G, Angeletti A, Hallak C. The mycoflora and toxicity of feedstuffs from a production plant in Córdoba, Argentina. *Mycotoxin Research*. 2002;18:8-22.
7. Wu F. Global impacts of aflatoxin in maize: Trade and human health. *World Mycotoxin J*. 2015;8(2):137-42.
8. Bueno DJ, Silva JO, Oliver G. Mycoflora in comercial pet foods. *Journal of Food Protection*. 2004;64:741-743.
9. Creppy EE. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters*. 2002;127:18-28.
10. Yu J, Chang PK, Ehrlich KC, Cary JW, Bhatnagar D, Cleveland TE, et al. Clustered pathway genes in aflatoxin biosynthesis. *Applied and Environmental Microbiology*. 2004;70:1253-1262.
11. CAST (Council for Agricultural Science and Technology). *Mycotoxins: Risks in plant, animal and human systems*. CAST. Ames, Iowa, USA; 2003.
12. Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP. Modification of immune function through exposure to dietary aflatoxin in Gambian children. *Environmental Health Perspectives*. 2003;111:217-220.
13. Wilson DM, Payne GA. Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination of the crops. *The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance*. DL. Eaton and JD. Groopman. San Diego, CA, Academic Press, Inc. 1994;309-325.
14. Hell K, Fandohan P, Bandyopadhyay R, Cardwell K, Kiewnick S, Sikora R, et al. Pre- and postharvest management of aflatoxin in maize. In Leslie, J.F, Bandyopadhyay R, Visconti A, (Eds) *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade*. CABI Publishing, Wallingford, UK. 2008;210-219.
15. Strosnider H, Azziz-Baumgartner, Banziger M, Bhat RV, Breiman R, Brune MN. Workgroup report: Public health strategies for reducing aflatoxin exposure in developing countries. *Environmental Health Perspectives*. 2006;114:1898-1903.
16. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *Am J Clin Nutr*. 2004;80(5):1106-1122.
17. Cotty PJ. Comparison of four media for the isolation of *Aspergillus flavus* group fungi. *Mycopathologia*. 1994;125:157-162.

18. Hsieh D. Potential human health hazards of mycotoxins. In "Mycotoxins and phytotoxins" eds. S. Natori, K. Hashimoto, and Y. Ueno. Third Joint Food and Agriculture Organization /W.H.O./United Nations Program. International Conference of Mycotoxins. Elsevier, Amsterdam. The Netherlands. 1988;69-80.
19. Lillehoj EB, Wall JH. Decontamination of aflatoxin-contaminated maize grain. In "Aflatoxin in Maize" eds. M. S. Zuber, S. B. Lillehoj and B. L. Renfro. A Proceedings of the Workshop, CIMMYT. 1987;260-279.
20. Klich MA. Identification of common *Aspergillus* Species. Centraal bureau voor Schimmecultures, Utrecht, Netherlands; 2002.
21. Groopman JD, Donahue KF. Aflatoxin, a human carcinogen: Determination in foods and biological samples by monoclonal antibody affinity chromatography. Journal AOAC International. 1988;71:861-897.
22. Atehnkeng J, Ojiambo PS, Donner M, Ikotun T, Sikora RA, Cotty PJ. Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three ecological zones in Nigeria. Int. J. Food Microbiol. 2008;122:74-84.
23. Gachara GW, Nyamache AK, Harvey J, Gnonlonfn GJB, Wainaina J. Genetic diversity of *Aspergillus flavus* and occurrence of aflatoxin contamination in stored maize across three agro-ecological zones in Kenya. Agric & Food Secur. 2018;7:52. DOI:<https://doi.org/10.1186/s40066-018-0202-4>
24. Northolt MD, van Egmond HP. Limits of water activity and temperature for the production of some mycotoxins. pp. 106-108. In G. A. Pepin, D. S. P. Patterson, and D. E. Gray (eds.), Proc. 4th meeting mycotoxins in animal disease. Weybridge, England; 1981.
25. Diener UI, Cole RJ, Sanders TH, Payne GA, Lee SL, Klich ML. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Ann. Rev. Phytopathol. 1987;25:249-270.
26. Fandohan P, Gnonlonfin B, Hell K, Marasas WF, Wingfield MJ. Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. Int J Food Microbiol. 2005;99(2):173-183.
27. Hell K, Cardwell KF, Setamou M, Poehling HM. The influence of storage practices on aflatoxin contamination in maize in four agro-ecological zones of Benin. West Africa Journal of Stored Products Research. 2000;36:365-382.
28. Udoh JM, Cardwell KF, Ikotun T. Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. Journal of Stored Products Research. 2000;36:187-201.

© 2018 Sowley et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history/26789>