IAA Journal of Scientific Research 12(2):71-75, 2025. www.iaajournals.org

©IAAJOURNALS ISSN: 2636-7319

https://doi.org/10.59298/IAAJSR/2025/1227175.00 IAAJSR:122

Mycoflora and Total Aflatoxins Determination in Spices Consumes in Mubi North Local Government Area of Adamawa State, Nigeria

[1]Isah Labaran, [2]Abubakar Isa and [3]Ibrahim U. Kashim

[1-3]Department of Science Laboratory Technology, Federal Polytechnic Mubi, Adamawa State, Nigeria
Corresponding email: isalkogobir@gmail.com

ABSTRACT

Aspergillus species are found all over the globe and may grow on a broad range of surfaces. Many species may cause food and spice degradation, as well as the development of aflatoxins, which is a public health concern in Nigeria and other tropical regions across the globe. Four species of Aspergillus were identified in four culinary spices regularly eaten in Adamawa state's Mubi north local government area. Spice samples included turmeric, garlic, ginger, clove, and nutmeg. The agar plate technique was utilised for inoculation and identification of Aspergillus species. Spice samples were inoculated on ampicillin-modified potato dextrose agar and cultured for seven days at 28°C to isolate mixed fungus colonies. The following aspergillus species were isolated in spices: garlic (Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, and Aspergillus parasiticus), nutmeg (Aspergillus fumigatus and Aspergillus parasiticus), ginger, and clove (Aspergillus niger and Aspergillus flavus), and tumerric (all species). The technique used for the testing of aflatoxins is ELISA process, therefore the total aflatoxins discovered are turmeric 0.13, clove 0.052498, ginger 0.052962, garlic 0.059992, and nutmeg 0.035. The obtained results show that the analysed spices eaten in Mubi North Local Government in Adamawa State provide low or no public danger. Given the significant impact of the toxins, there is a need for enhanced spice handling, preservation, and storage to reduce fungal infection.

Keywords: Spices, Aflatoxins, Aspergillus species and Fungi

INTRODUCTION

Aflatoxin are poisonous compounds generated by different types of toxigenic fungus known as mycotoxins. The discovery of aflatoxin dates back to 1961, after a significant epidemic of Turkey sickness in England. This resulted in the deaths of over 100,000 turkeys and other agricultural animals. Aflatoxins are the most lethal mycotoxins, produced by Aspergillus species, and are known to be one of the most lethal carcinogens due to the negative effects they can have on their consumers, as confirmed by the International Agency for Research on Cancer (IARC). They also stated that there is sufficient evidence in humans for the carcinogenicity of naturally occurring aflatoxin [1]. Two kinds of Aspergillus fungus are the primary producers of aflatoxins [2]. These are particularly prevalent in hot and humid climates. Asprgillusf lavus is omnipresent, preferring the aerial portions of plants (leaves, flowers), and generates B aflatoxins. Aspergilus parasiticus, which generates both B and G aflatoxins, is better suited to the soil environment and has a more restricted spread. Aflatoxins (mycotoxin) are a class of harmful secondary metabolites generated by certain strains of fungus under favourable conditions on a range of commodities. These natural food pollutants may be found in a variety of culinary spices and drinks, including garlic, ginger, nutmeg, beer, juice, and cloves. The majority of mycotoxins are generated by moulds such as Aspergillus flavus and Aspergillus niger. Mycotoxin-producing fungus may thrive under improper conditions of growth, harvest, transportation, and storage [3]. The most common kind of mycotoxin is aflatoxin, which is produced by Aspergillus flavus and Aspergillus paraciticus. The four primary forms are Aflatoxin B1, B2, G1, and G2, with M1 and M2 being metabolites of B1 and B2. In certain regions of Africa, the mycotoxin threat is quite high because a restricted food supply has led people to accept any materials that may be used as food, even if the food's quality has been altered by moulds. Furthermore, widespread malnutrition makes individuals more vulnerable to even low amounts of mycotoxin. The mycotoxin issue is exacerbated by warm, humid tropical circumstances, as well as poor drying and storage [4]. Also, practices that provide perfect circumstances for mould development and consequent

mycotoxin buildup in a short period of time. Aflatoxins are linked to a high prevalence of liver cancer in Africa and elsewhere, and they have been shown to worsen disorders such as Hepatitis B virus-induced and liver cancer [4]. Other health impacts of Aflatoxin in animals and humans include lower growth rate, weakened systems, and mortality. Thirteen (13) distinct forms of Aflatoxin are created naturally, with Aflatoxin B1 being the most poisonous and hence of significant public health relevance [5]. Aflatoxins are a group of toxics generated by specific fungus found on agricultural crops. Mould produces a poisonous substance that may harm the liver and often leads to liver cancer. Fungi that develop in sensitive goods used as a flavouring or condiment are often derived from particular plants such as seeds, leaves, bark, and roots [6]. They are mostly used to enhance the flavour of the dish. Spices are named from the term spiceses, which was used to describe a category of hazardous meals in the Middle Ages [5]. Spices are items made from diverse plant components that are often contaminated with fungal [5]. However, since a minor quantity of spices is present in food, they are regarded a key microbial carrier that is responsible for contamination owing to growth [8]. The primary contamination of fungal species that are detected as commensals on plants during surviving drying and storage [3]. Soil and air are the primary inoculum sources responsible for the contamination of crude spices in fields. Four locally accessible culinary spices are regularly used in food preparation in Mubi North Local Government Area, Adamawa State, Nigeria. Moisture levels identified in some of the food ingredients utilised in this investigation may have contributed to the development of microorganisms, which then produced aflatoxin [9]. As a result, this research is being conducted with the goal of isolating and identifying aflatoxin-producing fungus on spices used in Mubi North Local Government Area, Adamawa State. This study aims to isolate and detect aflatoxins-producing fungus in spices eaten in Mubi, North Adamawa State.

MATERIALS AND METHODS

Sample Collection

A total of four dried sample: ginger, garlic, clove and nutmeg were purchased from different places of famous Mubi north market in Adamawa State Nigeria during the month of May and June of 2019. The food spices were taking to the research Laboratory in the Department of Microbiology Modibbo Adama University of Technology Yola for preparation.

Media Preparation

The method of [10], 39.5 grams of potato dextrose agar was weighed and poured to 1000cm^3 conical flasks that contained distilled or de-ionized water. The flask was allowed to stand on the work bench for about 10- 15 minutes. It was then boiled to dissolve; the mouths of the flask were then plugged with non-absorbent cotton wool and finally cover with aluminum foil paper and masking tape. The medium was then sterilized by autoclave at the temperature of 121^{o}c per 1.1 kg for 15 minutes. It was then allowed to cool down to temperature of 40-45C, 250mg of ampicillin capsule was then added to the media to inhibit the growth of bacteria. then media was then poured in an aliquot amount of 20mls per plate allow to be solidified and kept at 40^{o}c for onward isolation of aflatoxin producing fungi from four (4) food spices samples were tested in this experimental study

Inoculation Procedure

An inoculums size of each of the four (4) food spices samples were then inoculated in four(4) plates each of Ampicillin modified potato dextrose agar aseptically, they were then incubated at the temperature of 28^{O}c for a period of seven (7) days for both mixed and pure colonies from the isolation of *Aspergillusniger*, *Aspergillus parasiticum*, *Aspergillus flavus and Aspergillus fumigatus* that were subjected to liquid broth culture of Ampicillin modified Potato dextrose broth incubated for seven(7)days at the temperature of28^{O}c for spectrometry analysis for the detection of aflatoxin production rate/level per each of the food spices samples which will be recorded as the rate of aflatoxcin production rate of each of the test food spices samples measured in Pcc per sample.

Aflatoxins determination

Sample Preparation

Two (2g) each of the ground samples was weighed into a container. Ten (10mL) of 70% methanol was added to the sample and mixed for 10 minutes using a shaker. The mixture was centrifuged at 3000 rpm for 10 minutes. A 100µl of the upper layer was mixed with 600µl of distilled water.

ELISA Procedure

50µl each of the diluted sample and standards were added to the ELISA plate wells.50µl of the conjugate was added to each well. 50µl of antibody was added to each well, mixed gently and incubated for 30 minutes at room temperature. The liquid was poured out of the wells and tapped vigorously against absorbent paper three times in a row to ensure removal of liquid from the wells. Wells were filled with 250µl of buffer and poured out again. A 100 µl of substrate was added to each well and incubate for 15 minutes at room temperature. A 100µlof stop solution was added to each well, mixed gently by shaking the plate manually and the absorbance measured at 450nm within 30 minutes.

RESULTS AND DISCUSSION

Table 1: Fungal Species Identified

SAMPLE	Common name	*A.niger*	*A.flavus*	*A. fumigatus*	*A.parasiticus*
(Zingiber officinale (ROSCON)	Ginger	+ve	+ve	+ve	+ve
(Allium Sativum (L.))	Garlic	+ve	+ve	-ve	-ve
(monodora mysristica (gaetan))	Nutmeg	+ve	+ve	-ve	-ve
(Eugenia aromatic (L.)	Clove	-ve	-ve	+ve	+ve
Curcuma longa L.	Turmeric	+ve	+ve	+ve	-ve

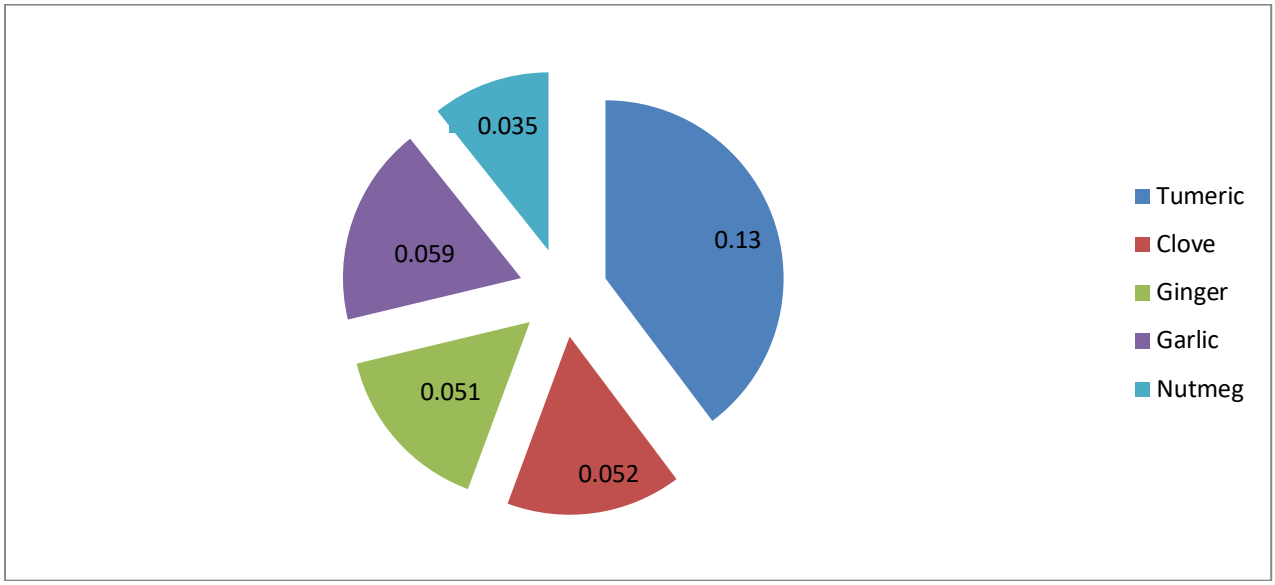

Figure 1: Total aflatoxins in spices (µg/kg)

The findings of this investigation revealed the presence of Aspergillus species in several commercial spices marketed in Mubi North Local Government Area, Adamawa State. The Aspergillus species content and concentration in four spices (Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, and Aspergillus parasiticus) in four locally available spices commonly used in spice preparation in Mubi North were garlic, ginger, clove, and nutmeg, and the results showed that all (100%) of the spices sample were contaminated with Aspergillus species. It was also discovered that, with the exception of garlic, which has the highest presence of Aspergillus (Aspergillus niger, Aspergillus flavus, Aspergillusfus migatus, and Aspergillus parasiticus), ginger, clove, and nutmeg have the lowest presence of Aspergillus species. Clove and ginger contain two Aspergillus species (Aspergillus niger and Aspergillus flavus), while nutmeg contains two Aspergillus species (Aspergillus fumigatus and Aspergillus parasiticus). This conclusion or observation is consistent with the findings of earlier investigations [11, 12]. However, levels of Aspergillus species lower than those identified in the current research have been recorded in several foods [13]. The existence of Aspergillus species in some of the spices utilised in this research might be related to many biotic and abiotic factors such as temperature, relative humidity, moisture content, spice chemical composition, storage time, and insect assault. According to [14], the optimal temperature for fungal development is 25-30oC with a relative humidity of 97-99%. Also, [15] previously stated that if both temperature (20-38oC) and moisture (16-24%) are suitable to Aspergillus species, aflatoxin may be generated within 48 hours. The moisture content of some of the culinary spices utilised in this investigation may have contributed to the proliferation of microbes and subsequent aflatoxin formation. [9] shown that moisture influences whether bacteria

may colonise a substrate or not. The high moisture content of several of the culinary spices utilised in this research might be attributed to the tropical and subtropical climates' high humidity (>70%) and temperature (>25%). [16]. In this research, garlic had the greatest prevalence of Aspergillus species due to its high moisture content, while clove, ginger, and nutmeg had the lowest presence due to the moisture and oil in them. The most prevalent genus found in the tested spices was Aspergilli. The high occurrence and higher levels of Aspergillus contamination in spices marketed in Mubi north may be linked to warm and humid climatic conditions that promote the development of toxigenic fungus [17]. The atmosphere offers perfect circumstances for mould development and subsequent buildup of mycotoxin in spices within a short period of time since spices provide a natural substrate for the growth of mould. Furthermore, agricultural techniques and postharvest handling of spices may lead to contamination by toxic fungus. It has been found that incorrect storage conditions encourage mould development and Aflatoxin contamination [18]. The total aflatoxins found include turmeric (0.13 µ/kg), clove (0.052498 µ/kg), ginger (0.052962 µ/kg), garlic (0.059992 µ/kg), and nutmeg (0.035 µ/kg). The results suggest that turmeric has the greatest content among the samples, while nutmeg has the lowest. The quantity of toxins found in turmeric may be the consequence of improper storage and treatment [19]. The spices sample are very vulnerable to mycotoxins, particularly when moisture and humidity levels are high, since aflatoxins formation is reduced at lower temperatures [19]. Because of the harmful nature of mycotoxins, various countries and international organisations, such as the FAO and NAFDAC, have formed food regulatory bodies in Nigeria. The results show that the examined spices have a low level of toxins. This suggests that there is no danger to public health.

CONCLUSION

The study concluded with the isolation and identification of aflatoxin-producing fungus in spices eaten in Mubi North, Adamawa State. The results show that Aspergillus species are isolated from the four spices, with garlic showing the presence of all species of Aspergillus (Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, and Aspergillus parasiticus), clove and ginger showing the presence of two species of Aspergillus (Aspergillus niger and Aspergillus flavus), and nutmeg showing the presence of two species of Aspergillus (Aspergillus fumigatus and Aspergillus parasiticus) during the studies. Aflatoxins in spices were measured as follows: turmeric 0.13 µ/kg, clove 0.052498 µ/kg, ginger 0.052962 µ/kg, garlic 0.059992 µ/kg, and nutmeg 0.035 µ/kg, with turmeric having the highest and nutmeg having the lowest. The obtained results show that the analysed spices eaten in Mubi North local government in Adamawa state provide low or no public danger.

RECOMMENDATION

1. Proper post-harvest processes, including drying and storage, may reduce fungal development and avoid mycotoxin contamination.
2. After identifying the aflatoxins-producing fungus and total aflatoxins, we propose further determining aflatoxins A1, B1, AFG, and others in the spice group. Consumed in Mubi.

Acknowledgement

We wish to acknowledge the tertiary education trust fund (TETFund) for funding this research under the institutional based research intervention and sincere appreciation tn the management of the federal polytechnic for their facilitation in approving and funding this research.

REFERENCES

1. Mostafavi SA, Akhondzadeh S, Mohammadi MR, Eshraghian MR, Hosseini S, Chamari M, Keshavarz SA. The reliability and validity of the persian version of three-factor eating questionnaire-R18 (TFEQ-R18) in overweight and obese females. Iranian journal of psychiatry. 2017 Apr;12(2):100.
2. Akiyamact, H., Goda, Y., Tanaka, T. and Toyoda, M. (2001) "Determination of Aflatoxins B1, B2, G1 and G2, in spices using a multifunctional column clean-up".Vol.932, pp153.
3. Kneifel and Berger (1994)."Microbial criterialof random samples of spices and herbs retailed on the Austrian market" J. Food protect. 57:893-901
4. Bankole and Joda (2014)."effects of lemon grass (cymbopagan citrus stap) powder and essential oil on mould detororation aflatoxin contamination of melon seeds (*Colocynthiacitrus*) *African Journal of Biotechnology 3(1):52-59*
5. Mckee (1995)."Microbial contamination of spices and herbs: *A Review" Lebensm.*Wiss technology: 28:1-11.
6. Adebayo and Oladopi,(2006). "They are used mainly for enhancing task taste of the food" *African Journal of Biotechnology, 2:129-135.*
7. Jimoh and Kolapo (2008)."Mycoflora and Aflatoxin production in market samples of some selected Nigerian foodstuffs. *Research Journal of Micro biology, 3:168-174*
8. Sharma, A.K,Ghanekar, R.s., Podwal-Desai and Nadkami (1985). "Studies on seed mycoflora of some umbeliferousspices".*Journal of AgriculturalScience Digest;* 4(2):71-73.

9. Smith and Moss (1995)."Mycotoxius formation analysis and Significance wiley and son Cliclester".*Journal of food research.*(P.148)
10. Juliana C, Fernandes-Alnemri T, Wu J, Datta P, Solorzano L, Yu JW, Meng R, Quong AA, Latz E, Scott CP, Alnemri ES. Anti-inflammatory compounds parthenolide and Bay 11-7082 are direct inhibitors of the inflammasome. Journal of Biological Chemistry. 2010 Mar 26;285(13):9792-802.
11. Adebayo – Tayo, B.G Onilude, A.A, Ogunijobi, A. A., Gbolagade, J. S. and Oladapo (2006)."Detection of fungi and aflatoxinin shelved bush mango seed (Irvingia SP) stored for sale in Uyo, Niger". *African Journal of Biotechnology, 5:1729-1732.*
12. Romagnoli, B., Meena, V., Gruppion, N, and Bergamini, C. (2007)."Aflatin spices, aromatic herb teas and Medicinal plants market in Italy". Food control.18:697-01.
13. Zinedine, A;Brera, C., Elakudari, S. Catanoc.Debegnach, S. Anglini, B., Desantsantis, Faid, M. Benlemlih, V. Minardi and Miraglia, (2006)."Natural occurance of mycotoxins in cereals and spices commercializes in Morocco".*Jounal of Food control,* 17:868-874.
14. Simsek, (2002)."mycoflora of hazelnut (coryusavellana L.) andaflatoxin content in hazelnut kernels artificially infected with Aspergillusparasiticus". Nalirung*Journal of food research.*46:194-196.
15. Rose, I. (1979). "Potential for Aflatoxin development in low temperature drying system". *American Society of Agricultural Engineers paper 2:12-18.*
16. Howard GT. Biodegradation of polyurethane: a review. International Biodeterioration & Biodegradation. 2002 Jun 1;49(4):245-52.
17. Adzahan and Jalilin (2009)."Survey of Aflatoxin in retail samples of whole and ground black and white peppercorns.Food Additcontam". *journal of scientific research and reports. 2(2):178-182*
18. Rocha and Fonseca (2008)."Distribution of fungi and aflatoxins in a stored peanutvariety".*Journal of scientific research and reports.* 106:285-290
19. Sharfa F. K. Clarence M. and Kessy F. K. (2024). Aflatoxins contamination in spices in selected area of Tanzania and their detection by chromatographic technique. African journal of health sciences. 24(2). 140-151.

CITE AS: Isah Labaran, Abubakar Isa and Ibrahim U. Kashim (2025). Mycoflora and Total Aflatoxins Determination in Spices Consumes in Mubi North Local Government Area of Adamawa State, Nigeria. IAA Journal of Scientific Research 12(2):71-75. https://doi.org/10.59298/IAAJSR/2025/1227175.00