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Preliminary screening of toxigenic fungi and mycotoxin contamination: A case of agricultural products in Ivory Coast

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ABSTRACT

Toxigenic fungi are generally known to contaminate agricultural products; the causes, rate, and extent of its effects on common products have less emphasis, especially in sub-Saharan African countries. This lack of emphasis could be attributed to the state of research, storage, and accountability that could have intimated the public with necessary measures to curb the menace.

For the purpose of the food safety control, this study investigates the fungal presence and mycotoxins in major food crops such as cereals (millet, rice, white and yellow maize), vegetables (chili, gnangnan, haricot, melon, and okra), legumes (haricot), and root products (attieke, cassava flakes) in Abidjan, Ivory Coast. Each sample is serially diluted and inoculated onto Czapek yeast agar (CYA), malt extract agar (MEA) and potato dextrose agar (PDA), to screen for fungal contamination. Colony color, colony edge, mycelia color, conidia shape, and microscopic features are used to identify the isolates. In addition, thin layer chromatography (TLC) was used to identify seven mycotoxins in food commodities. It is revealed that, *Aspergillus* species are found in 64 (92%) of the samples, *Penicillium* species in 29 (41%), and *Fusarium* species in 20 (29%) of the samples. A significant incidence rate of aflatoxin B_1 (AFB_1) is found in vegetables (gnangnan, haricot, and melon), roots products (cassava flakes), cereals (rice, and white maize). According to this investigation, each sample contains one or more fungus and mycotoxins.

1. Introduction

Fungi contaminate agricultural products, causing various changes during growth in the field and after harvest (Marin et al., 2021). This is because fungi inhabit almost every niche in the environment. The effects of fungal infection on agricultural products include discoloration and unpleasant odor, chemical and nutritional changes, and loss of quality (Rawat, 2015). The presence of toxigenic fungi on plants affects productivity, value and quality (Bennet & Cahill, 2016), and they are also responsible for the production of various mycotoxins that have negative effects on human and animal health (Marin et al., 2021; Adekoya et al., 2018). Intake of these mycotoxins by animal or human can result in acute or chronic illness all of which are collectively referred to as mycotoxicosis, a term describing the disease caused by mycotoxins (Tripathi & Alam, 2020). Acute toxicity occurs when exposed to a large dose in a short period of time, and it can cause a disease leading to death in some situations (Barac et al., 2019). Long-term exposure (chronic toxicity) can harm the immune system (modifying human immunity), the neurological system, and cells, and can even cause cancer in humans (IARC, 2015; Shahba et al., 2021; Campbell & Weinstock 2022). Mycotoxins (aflatoxins) have been associated with liver damage and cancer in humans and animals. In both human and animal cells, mycotoxins have a strong propensity and capacity to enter the cellular genome where they cause a significant mutagenic change in the nucleotide sequence that result in strong and irreversible defects in the genome (Adam et al., 2017). This defect will eventually be transcribed and translated, resulting in the growth of cancer because most of these toxins especially the aflatoxins are procarnogenic that were transformed *in vivo* to their carcinogenic derivatives (Groopman et al., 2021).

Mycotoxins are mostly produced by *Aspergillus, Fusarium,* and *Penicillium* species (Tripathi & Alam, 2020) Food commodities affected by toxigenic fungi and mycotoxins includes cereals, cereals products, beans, yam, cassava, peanut, spices and chilies (Bouseta et al., 2021; Imade et al, 2021; Okungbowa & Kinge, 2021; Aasa et al., 2022a). Aflatoxins (AFs), ochratoxins (OTs), zearalenone (ZEA), fumonisins (FBs), and deoxynivalenol (DON) are some of the most common mycotoxins found in food, and reports show that more than 25% of the world's agricultural products are affected by them (Marin et al., 2013; Eskola et al, 2020; Luo et al., 2021) with frequent disastrous consequences on food security and safety.

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Environmental factors such as high temperature and humidity increase the risk of fungal growth and mycotoxin production (Daou et al., 2021), therefore the climatic conditions of Ivory Coast which is characterized by high temperature and high relative humidity are favorable for fungal growth; thus fungal presence in their agricultural products is conceivable. The risk associated with these microbes and their metabolites in food make it necessary to always to screen for their presence. Therefore, this study, focus on major toxigenic fungi and their mycotoxins because identification is an important step towards their control and reduce their economic and health in the society.

2. Methodology

2.1. Sample collection and treatment

In this investigation, 70 food commodities were selected at random from Adjame, Yopougon, and Cocody in Ivory Coast. During the sampling process, samples were collected based on the availability of the product. Some of the samples include cereals (millet, rice, white and yellow maize), vegetables (chili, gnangnan, haricot, melon, and okra), legumes (haricot), and root products (attieke, cassava flakes). Individual samples weighing approximately 100 g were placed in zip-lock bags. Each sample was thoroughly mixed before being selected to obtain a representative sub-sample of the food commodities. They were then transported to the mycological laboratory in South Africa. The sample was ground into smaller particles and was then kept at -8°C until further analysis.

2.2. Mycological analysis

The approach described by Aasa et al. (2022a) was used for fungal isolation. Exactly, 1 g of each sample was weighed into a test tube containing 10 mL of sterile Ringer's salt solution and vortexed for culture preparation. After that, each sample was serially diluted until it reached a concentration of 10^{-5}. Each mixture was inoculated onto potato dextrose agar (PDA) (potato extracts 4 g/L, dextrose 20 g/L, and agar 15 g/L), Czapek yeast agar (CYA), and malt extract agar at concentrations of 10^{-3}, 10^{-4}, and 10^{-5} (MEA). The inoculated plates were then incubated for 5–7 days at 25°C.

2.3. Fungal identification

To identify fungal cultures on CYA, MEA and PDA, morphological properties such as colony structure, color, formation growth pattern, spore morphology, and formation of distinctive conidiophores were used (Klich, 2002; Pitt & Hocking, 2009). Pure colonies were identified further by mounting mycelium on slides and stained with lactophenol cotton blue. The slides were then examined under a 400 X magnification optical microscope (Olympus CX-40, micro-Instruments, New Zealand Ltd.).

2.4. Multi-mycotoxins extraction

The analysis followed the description provided by Malachova et al. (2015). Five grams (5 g) of ground food samples were emulsified in 20 mL of extraction solvent (acetonitrile/water/acetic acid, 79:20:1, v/v/v) and placed on a mechanical shaker (LABCON, GmbH, Heppenheim, Germany) for 90 min at 180 RPM and then centrifuged for 5 min at 3000 rpm using an Eppendorf Millipore Laboratory Centrifuge (Merck South Africa). Following that, the extracts were filtered through a 0.22 m particle size PTFE syringe filter and 500 mL were transferred into a 1.5 mL glass vial containing equal volumes of dilution solvent (acetonitrile/water/acetic acid, 79:20:1, v/v/v) and vortexed for 30 s. The filtrate was spotted on TLC plates after dilution, and the plates were subjected to the TLC outlined below.

2.5. Mycotoxin screening by TLC

A two-dimensional thin layer chromatography (TLC) approach was used to screen for AFs (AFB_1, AFB_2, AFG_1, and AFG_2), OTA, DON, and ZEA utilizing a silica gel-backed 20 × 20 cm plate (Sigma-Aldrich, Germany). The plates were faintly marked 10 mm from each edge across each corner, which aids in the measurement of the retardation factor (RF). 20 µl of the extracts were spotted about 10 mm from the edge of the silica gel TLC plate.

For certain mycotoxins such as AFs, DON, and OTA, the mobile phase used was dichloromethane/ethyl acetate/propan-2-ol (90:5:5 v/v/v) (DEP) for the first dimension and toluene/ethyl acetate/formic acid (6:3:1 v/v/v) (TEF) for the second dimension, while for ZEA, dichloromethane/acteone was used. All the mobile phases were transferred to the chromatographic tank; plates were inserted and allowed the solvent to reach the top of the plate before being removed from the tank. Plates were dried and observed under UV (ultra violet) light at a wavelength of 365 nm after each mycotoxin run. Chromotropic acid (CTA) and cold diazotized dianisidine were sprayed onto DON and ZEA plates, respectively. To identify the mycotoxins present, the colour of the toxin under UV and retardation factors of each spot on each plate were determined and compared to standards.

Control experiments were also carried out to assess the method in addition to using a validated method. Sensitivity, accuracy, and detection limits were established as the standard parameters for method validation. The limit of detection was determined visually; known concentrations of mycotoxins standards were prepared, successively diluted, and subjected to TLC until the minimum concentration at which the analytes can be detected was established and used as the limit of detection. In addition, the higher the concentration of mycotoxins, the more sensitive and bright they were under UV light.

3. Results and discussion

The mycobiota of food commodities from Ivory Coast revealed of many species belong to the *Aspergillus, Fusarium* and *Penicillium*. The results obtained include a macroscopic view of fungal colonies recovered from various food commodities (Figs. 1–3) using morphological characters such as colony color, colony edge, mycelia color, conidia shape, and microscopic characteristics.

3.1. Distribution of fungi in food commodities from Ivory Coast

Fig. 4 presents the rates of occurrence of the isolated fungus. The data revealed that 92% of the analysed samples were contaminated with *Aspergillus* species. In order of prevalence, the most contaminated food commodities with *Aspergillus* species include white maize, rice, okra, chili, melon, haricot, and gnangnan. The isolation of mycotoxigenic fungi and the predominance of the genus *Aspergillus* in food agree with the work done by Koffi-Nevry et al. (2013) and Adjovi et al. (2019). The genus *Penicillium* was also isolated with 41% of the samples contaminated, with the most contaminated samples including chili, haricot, melon, and rice. This survey also revealed the occurrence of *Fusarium* species in the analysed samples. As shown in Fig. 2, 29% of the tested samples were contaminated with millet (66%), white maize (50%), and haricot (50%). The study shows that toxigenic fungi especially *Aspergillus* and *Penicillium* are ubiquitous in this region and that this may be as a result of climatic conditions favorable for fungal growth, harvesting techniques, and post-harvest storage. According to Pitt and Hocking (2022), temperature, water activity, and pH influence the growth of fungi in foods. Other factors that controlled the fungal growth include light and nature of substrate (Mannaa & Kim, 2017).

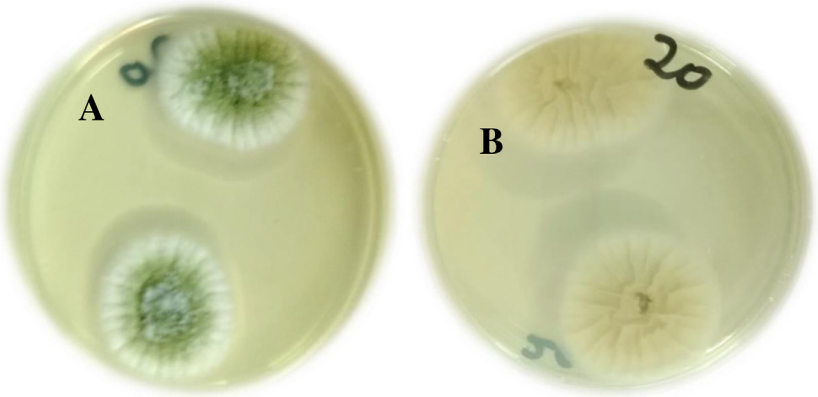

Fig. 1. Macroscopic features of *Aspergillus flavus* isolated on CYA media (A: Front; B: Reverse). The isolates produce olive green conidia in five days, flat and smooth at the verge then elevated at the middle and furrowed in a cerebriform shape. The colonies were enclosed in white mycelium. The reverse is slightly pale.

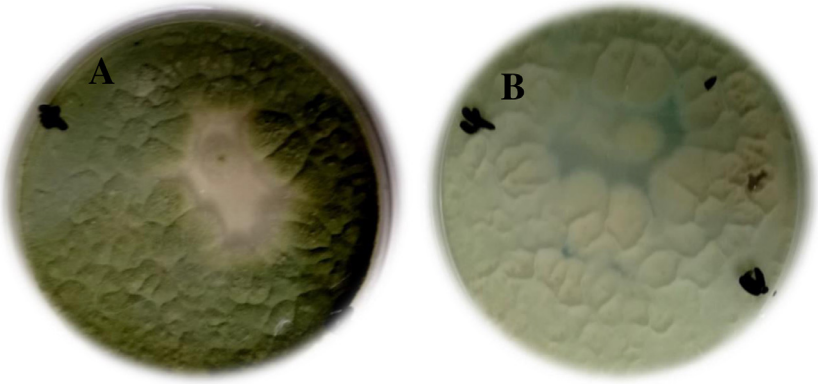

Fig. 2. Macroscopic characteristics of isolated *Aspergillus fumigatus* on CYA media (A: Front; B: Reverse). The isolates produce a turquoise color in five days, slightly wrinkled and dense. It contains floccose growth and mycelium inconspicuous. The reverse is pale.

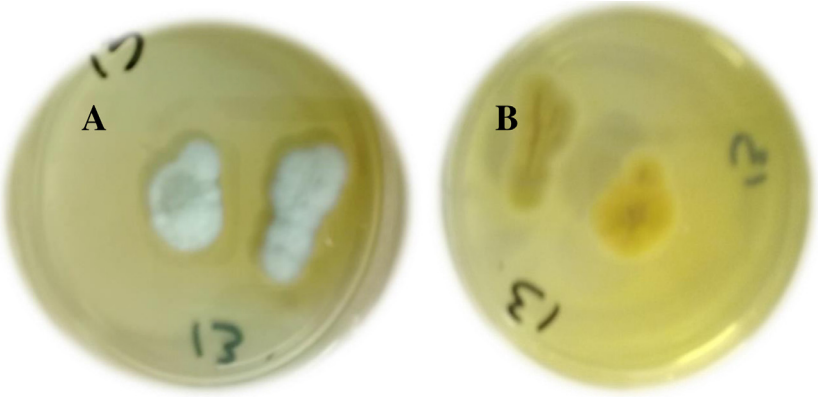

Fig. 3. Macroscopical characters of isolated *Aspergillus candidus* on CYA media. (A: Front; B: Reverse). Colonies grow slowly, and white colored, mycelium inconspicuous, reverse is pale.

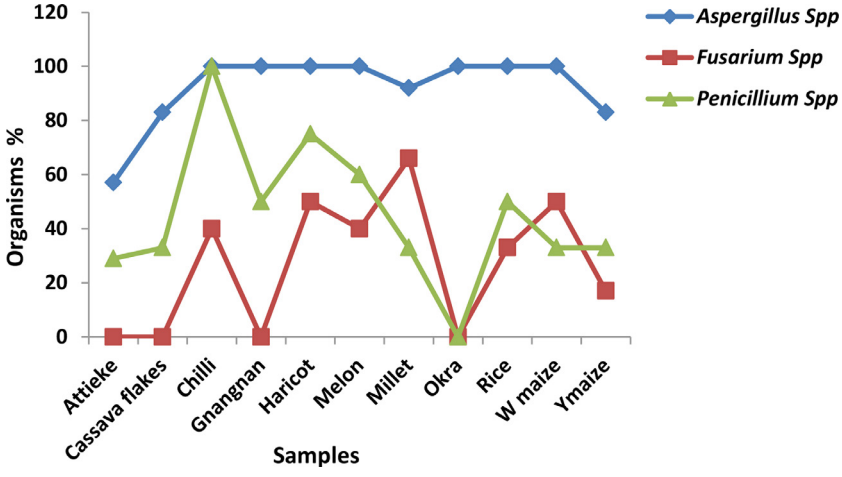

Fig. 4. The proportion of samples contaminated with fungi.

Table 1
Method validation parameters.

Mycotoxins	Retardation factors (RF_1 and RF_2 mm)	LOD (µg/kg)
AFB_1	57–61	0.50
AFB_2	55–56	1.00
AFG_1	55–57	2.50
AFG_2	51–53	2.50
OTA	57–76	10.0
DON	38–57	100
ZEA	28–79	20.0

AFB_1 - aflatoxin B_1; AFB_2 – aflatoxin B_2; AFG_1 – aflatoxin G_1; AFG_2 - aflatoxin G_2. OTA - ochratoxin A; DON - deoxynivanenol; ZEA - Zearalenone. LOD - Limit of detection.

3.2. Mycotoxin analysis

Extracted mycotoxins were analysed using chromatographic techniques. The present study evaluates the quantitative measure of mycotoxin in food in the food sample using TLC. The AFs, DON, ZEA, and OTA presence were confirmed in the samples. Limit of detection for AFB_1, AFB_2, AFG_1, and AFG_2 were 0.50, 1.00, 2.50, 2.50 µg/kg on TLC, respectively. OTA, DON and ZEA limit of detection recorded were 10, 100 and 200 µg/kg, respectively (Table 1). TLC could not detect AFB1 at concentration less than 0.50 µg/kg, also for OTA, concentration below 10.0 µg/kg could not be detected.

The retardation factors of each spotted mycotoxin were circled, calculated, and compared with the standards in order to identify the toxins. The TLC employed in this research revealed the mycotoxins in food samples (Table 2; Fig. 5); however, quantification is challenging since this technique is often not sensitive enough for more detail analysis. Therefore, it is used specifically for initial screening and for accurate quantification; more sensitive and advanced techniques will be needed.

The fluorescence of AFs, OTA, DON, and ZEA viewed under the ultra violet light revealed that some samples were positive, showing a light blue fluorescence for AFB_1 and AFB_2, a light green for AFG_1 and AFG_2, and a blue-green fluorescence for OTA. All positive extracts for AFB_1, AFB_2, AFG_1 and AFG_2 were spotted within the range of 57-61 mm, 55-56 mm, 55-57 mm, and 51-53 mm (Fig. 5). In addition, the chemical structure of the detected mycotoxins in all the analysed samples is depicted in Fig 6.

The TLC data revealed that AFB_1 was the main contaminant with a 39% incidence rate, while AFB_2 (20%) and a 31% incidence rate for AFG_1 and AFG_2 were also recorded. The results revealed that haricot, melon, cassava flakes, gnangnan, and white maize were contaminated with AFB_1 with an incidence rate of 50% and above. The most prominent among aflatoxins was AFB_1 although a high incidence rate of AFB_2 was found in Gnangnan (Table 2). Yellow maize (50%) and millet (58%) were the analysed samples with a high incidence rate of AFG_1 and AFG_2 as shown in Table 2. Among the analysed samples, only melon was free of AFB_1. Other samples had a trace of AFB_1. However, no sample is totally free of aflatoxins if AFB_1 is not found. AFB_2, AFG_1 or AFG_2 will be found in them. Scientific researchers have reported the presence of aflatoxins in crops including maize, peanuts, chili, and spices (Gnonlonfin et al., 2013; Okun et al., 2015; Ponce-García et al., 2021).

Over five billion people are at risk of chronic exposure to aflatoxin-contaminated foods/feeds especially in developing countries (Ismail et al., 2019; Hassan et al., 2021). Maize, being one of the most highly consumed crops worldwide, is most susceptible to aflatoxin contamination, therefore making it the main source of human exposure to aflatoxin (Jallow et al., 2021; Ponce-García et al., 2021). Aflatoxins, especially AFB_1, have been classified as Group 1 carcinogens because it has been known that aflatoxin exposure causes liver cancer in humans and in several animal species (IARC, 2015) and the review showed that the danger of aflatoxicoses (a disease caused by aflatoxins) still exists in Africa (Amaranta et al., 2021).

For OTA, 34% incidence rate was recorded in this study. Food samples with the highest rate of contamination include chili (80%), haricot (50%) and white maize (50%). This is in accordance with reports by Ano Guy Serge et al. (2017) who recorded the presence OTA in dried maize, gumbo and cassava consumed in Ivory Coast. Based on sufficient evidence of its carcinogenicity in animals, OTA has been classified as group 2B, possible human carcinogen. OTA is a nephrotoxin that are potential carcinogenic, teratogenic, and immunosuppressive in nature (Mannaa &Kim, 2017).

Thirty-four percent of all the analysed samples were contaminated with DON. The chemical structure of the detected DON in the analysed samples is shown in Fig 6. The samples that were contaminated with DON included gnangnan, chili, and yellow maize, with an incidence rate of 83%, 80%, and 50%, respectively. Report shows that DON has been found in food commodities and is mainly produced by *Fusarium culmorum* and *Fusarium graminearum* (Ji et al., 2019). The effects of DON on humans and animals include vomiting thus it also referred to as vomitoxin. Other adverse effects of DON on humans and animals are digestive disorder, oxidative damage, and reproductive toxicities (Gerez et al., 2021).

TLC analysis of the food samples showed the presence of ZEA with an incidence rate of 27% in all the analysed samples. Haricot was found to be the most contaminated sample, with an incidence rate of 50%. However, okra was found to be free of ZEA. This showed that haricot is a favorable substrate for *Fusarium* species and the production of ZEA. Zearalenone is another mycotoxin produced by the various *Fusarium* spp including *Fusarium graminearum*, and *Fusarium culmorum* which induces liver lesions, hepatocarcinoma and alters hepatic function in animals (Pistol et al., 2014). According to Choi et al. (2012), zearalenone is nephrotoxic, hematotoxic, immunotoxic, hepatotoxic, and enhance lipid peroxidation.

Table 2
Incidence (%) rate of mycotoxin by thin layer chromatography.

Samples	No of analysed samples	AFB_1	AFB_2	AFG_1	AFG_2	OTA	DON	ZEA
Attieke	7	3(43)	0(0)	0(0)	0(0)	3(43)	1(14)	2(29)
Cassava flakes	6	4(67)	0(0)	0(0)	0(0)	1(17)	1(17)	1(17)
Chilli	5	1(20)	0(0)	0(0)	1(20)	4(80)	4(80)	1(20)
Gnangnan	6	5(83)	5(83)	0(0)	1(17)	2(33)	5(83)	2(33)
Haricot	4	2(50)	1(25)	1(25)	0(0)	2(50)	0(0)	2(50)
Melon	5	0(0)	0(0)	0(0)	1(20)	1(20)	1(20)	1(20)
Millet	12	4(33)	4(33)	3(25)	7(58)	4(33)	4(33)	5(42)
Okra	7	1(15)	0(0)	1(15)	0(0)	1(15)	2(29)	0(0)
Rice	6	2(33)	0(0)	0(0)	0(0)	1(17)	1(17)	1(17)
White maize	6	3(50)	2(33)	1(17)	2(33)	3(50)	2(33)	2(33)
Yellow maize	6	2(33)	2(33)	3(50)	1(17)	2(33)	3(50)	2(33)

No - number; AFB_1 - aflatoxin B_1; AFB_2 – aflatoxin B_2; AFG_1 – aflatoxin G_1; AFG_2 - aflatoxin G_2. OTA - ochratoxin A; DON – deoxynivanenol; ZEA - Zearalenone.

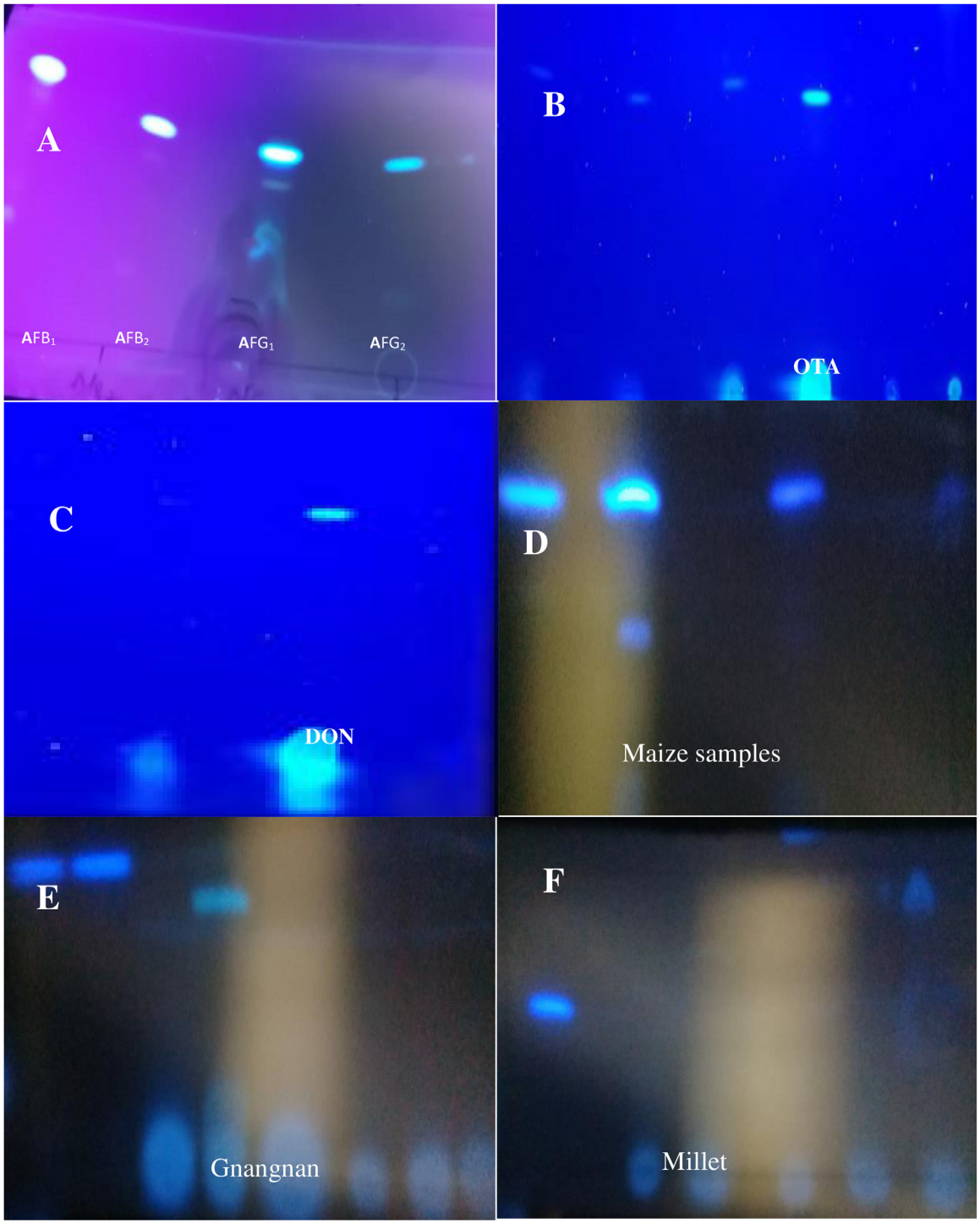

Fig. 5. View of some TLC plates under long wave ultra violet light showing the spot of mycotoxin standard and mycotoxin presence and extracted from food samples. One dimensional TLC: - A: Aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2), B: OTA standard, C: DON standard, D: Yellow maize, E: Gnangnan, F: Millet. The lanes with bands in D, E, and F, are the samples showing fluorescence as mycotoxin standards. The bands that were detected mean that the samples are void of mycotoxin.

Fungi and mycotoxin contamination of food in this region could be attributed to the favorable climatic conditions during production, storage, processing, transportation or marketing (Darwish et al., 2014). One of the most serious threats to processed and stored food products worldwide is the spoilage of food by toxigenic fungi (Gnonlonfin et al., 2013). In Sub-Sahara Africa the climatic conditions, including high temperature, aeration, and high relative humidity are all conditions that accelerate fungal development and mycotoxin production (James & Zikankuba, 2018; Lulamba et al., 2019).

The health consequences of consuming such contaminated food are enormous, and based on the data generated in this research, it is clear that Ivorian's are exposed to mycotoxins and thus face the risk of mycotoxicoses (diseases caused by mycotoxins). Mycotoxin contamination of food commodities in sub-Saharan Africa is not only dangerous

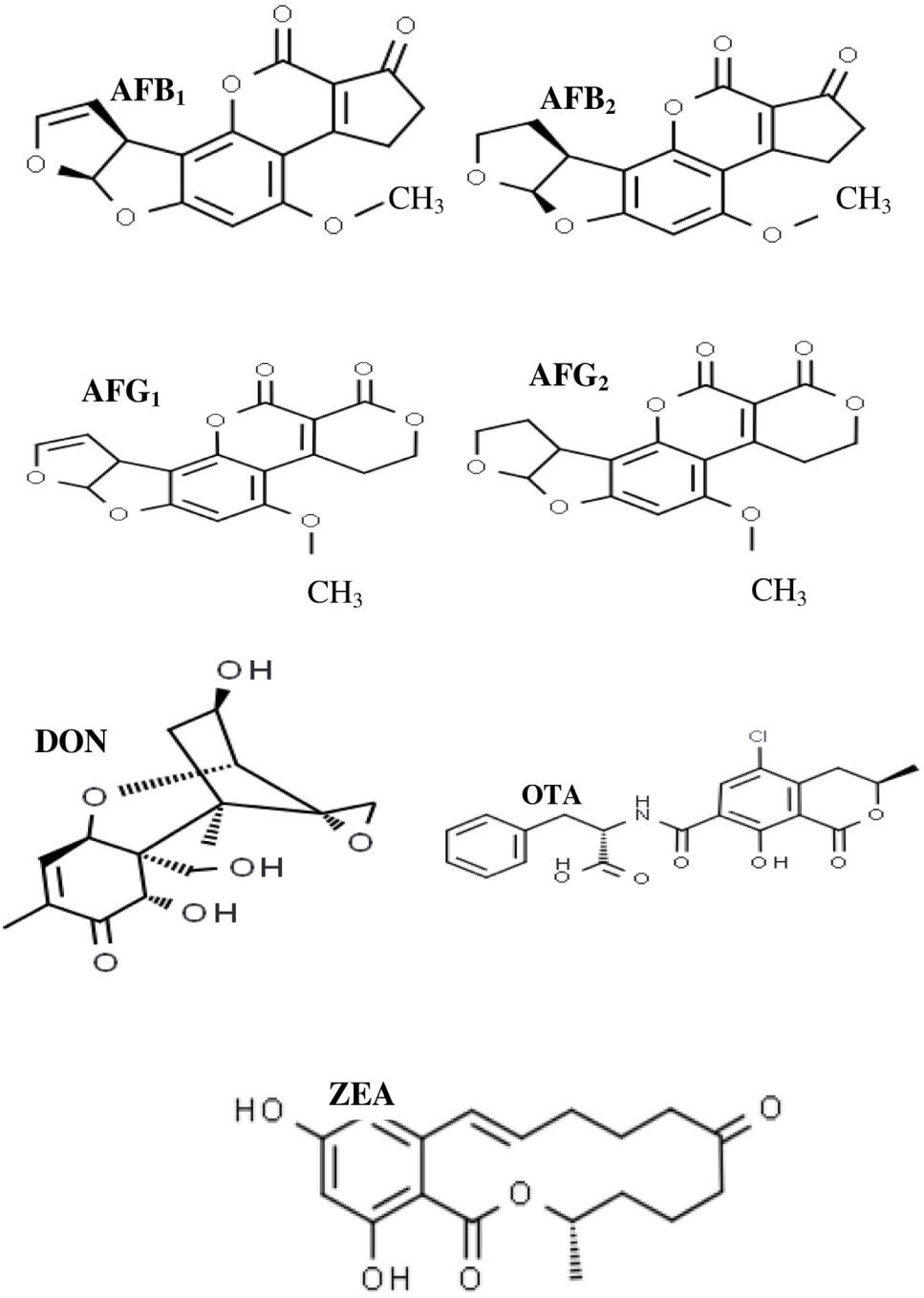

Fig. 6. Chemical structures of the detected mycotoxins in this study.

to healthiness, but also to trade and the country's growth. According to Aasa et al. (2022b), fungal and mycotoxin contamination in West African food products is rarely accessed and almost never controlled, as most of these countries don't have regulatory limits for mycotoxins thus limiting the exportation of agricultural products, which has an adverse effect on the economic growth and development of the country. The availability of safe food is a prerequisite for the well-being of people and the growth of the country's economy.

Therefore, to ensure a growing economy, food safety measures must be implemented to avoid the negative effects of this contaminant. Good agricultural practices, proper handling, appropriate processing techniques, cleaning, and adequate storage conditions can all help to reduce contamination levels. Food safety controls should be implemented at every stage of the manufacturing process.

4. Conclusion

The research showed the ubiquitous presence of the fungi and mycotoxins in the various food samples in Ivory Coast. The presence of *Aspergillus, Fusarium*, and *Penicillium* species describes the fungal diversity in food commodities. These fungi, especially *Aspergillus*, the prominent isolated genus in this study, have been reported in various food commodities, indicating that they are significant genus that should not be overlooked. The confirmation of AFs, OTA, DON and ZEA in some of the analysed samples shows that drastic steps must be taken to avert the colossal effects of these contaminants on food quality and quantity. Therefore precaution measure must be taken to reduce their in agricultural products in this country. TLC techniques could be used in absence of advanced techniques like high performance liquid chromatography

especially in less economically developed countries for early detection and cost management to screen for toxins to help the community to necessary action before it's too late while waiting for the advance techniques.

Credit author statement

The author confirms that Aasa A.O., and Njobeh P.B. conceived of the ideas and developed the research goals and objectives. The methodology used in this article was designed by Aasa A.O., Fru F.F., and Njobeh P.B., who also planned and executed the research activity. Fru F and Njobeh P.B encouraged and oversaw Aasa A.O's investigation into Fungi and mycotoxin contamination in food commodities. The findings were discussed by all authors, and they all contributed to the final manuscript. The activities to annotate, scrub data, and maintain research data were managed by Aasa A.O., Fru F.F., and Adelusi O.A. Aasa A.O assisted in the application of statistical, mathematical, and computational techniques to study data analysis. The experiments and data collection were carried out by Aasa A.O. Fru F.F., and Njobeh P.B., oversaw research activity planning and execution, as well as mentorship. Njobeh P.B. provided financial support for the research that resulted in this publication, as well as reagents, materials, instrumentation, and other analysis tools.

Verification of the research activity, experiments, and research outputs was assisted by Aasa A.O., Areo O. M.., and Adelusi A.O. Aasa A.O. drafted and prepared the manuscript for publication, and Areo O.M., Adelusi O.A., and Fru F.F. assisted in critical review, comment, and revision of the manuscript for important intellectual content (Fig. 6).

Declaration of Competing Interest

There is no conflict of interest

Data availability

The data that has been used is confidential.

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