Scientific Research Publishing

Advances in Microbiology, 2023, 13, 361-372
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Geographic Distribution of *Aspergillus* Section *Flavi* Subspecies Isolated from Crops, Foods, and Feedstuffs in Benin

Fossou Joli Prince Mintognissè[1,2], Adjovi Yann Christie Sissinto[1,2*], Ahehehinnou Hilarion Ulrich Mawuton[1,2]

[1]Laboratory of Biochemistry and Molecular Biology, ISBA, Cotonou, Benin
[2]Department of Nutrition and Food Sciences, National University of Agriculture, Ketu, Benin
Email: *yann.adjovi6@gmail.com, fossouprince@gmail.com, Mawutonahehehinnou4@gmail.com

How to cite this paper: Mintognissè, F.J.P., Sissinto, A.Y.C. and Mawuton, A.H.U. (2023) Geographic Distribution of *Aspergillus* Section *Flavi* Subspecies Isolated from Crops, Foods, and Feedstuffs in Benin. *Advances in Microbiology*, **13**, 361-372.
https://doi.org/10.4236/aim.2023.138023

Received: December 30, 2022
Accepted: August 4, 2023
Published: August 7, 2023

Copyright © 2023 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Mycotoxins are one of the most dangerous chemical contaminants found in staple foods. Aflatoxins, a well-known mycotoxin, are mostly dangerous for both humans and livestock, according to the International Agency Cancer for Research on Cancer. In this paper, the author aimed to establish a geographical distribution map of aflatoxins fungi producers on several food like maize, maize of popcorn, popcorm, fish, spices, cassava, mil, sorghum, peanut and voandzou from the eight (8) agroecological area in Benin. Several molds were isolated from these foods and morphologically characterized on malt extract agar. Among the fungi, 318 species isolated from four leading subspecies, *A. flavus*, *A. parasiticus*, *A. nomius,* and *A. togoensis,* belonging to the *Aspergillus* section *Flavi,* were identified. For molecular analysis, polymerase chain reaction (PCR) methods were used to amplify 1450 pb of the beta-tubulin gene, and their products were used to realize restruction fragment length polymorphisms (RFLPs) to identify genic characteristics. From this strain, three DNA fragments were identified as belonging to subspecies *A. flavus,* and four DNA fragments were classified as belonging to *A. parasiticus*. Furthermore, chemotaxonomic's analysis was performed on all strains isolated by thin layer chromatography revealing variable levels of aflatoxin's contamination in the samples. All feedstuffs from the eight agroecological areas in Benin were found to be contaminated by several molds, and their mycotoxins mainly comprised four types of aflatoxins: B1, B2, G1 and G2. This study revealed that the four agroecological areas in northern Benin are primarily contaminated by *A. flavus* and *A. togoensis,* producers of aflatoxins B1 and B2, whereas the four agroecological areas southern Benin are contaminated by *A. parasiticus* and *A. nomius,* producers of both sets of aflatoxins B1 and

B2 and aflatoxins G1 and G2. This study can be used to protect public health from the risks associated with liver cancer.

Keywords

Agroecological Area, Foods, Aflatoxins, TLC, PCR

1. Introduction

Benin, a developing country in West Africa, agriculture represents the basis of the Beninese economy. Although traditional, it provides populations with considerable income for their development. Each year, Government devotes a considerable part of its budget to improving productivity and limiting pre-and post-harvest losses. For that, Benin is divided into eight agroecological areas based on staple and feed commodity production [1]. During their growth, harvest, storage, transport, drying, selling, and transformation, raw materials are regularly contaminated by molds and their chemical hazards mycotoxins [2] [3].

Mycotoxins are toxic substances generally produced by molds. These substances resist at oxidation, acidity, heat, and cold [4]. Among the 400 known mycotoxins, aflatoxins are the most dangerous and the most researched, due to their high toxicity [5].

Aflatoxin is a poison produced by *Aspergillus* section *Flavi*. From this section, both *A. flavus* and *A. parasiticus* are the most dangerous species and are frequently found in crops, in food, and feed as they are plant pathogens that produce aflatoxins [6]. Among the more than 12 aflatoxins [5], B1, B2, G1 and G2 are particularly dangerous for both humans and livestock, due to their consequences [7]. In fact, aflatoxin B1 has been classified as a carcinogen by the International Agency for Cancer Research since 2002 [8].

Aflatoxin production can occur at any time. However, certain factors involving temperature, drought conditions, and heat are necessary [9]. High temperatures accentuate fungal and aflatoxic contamination [10]. Aflatoxins are resistant to heat destruction at temperatures that can be found in activities such as cooking, extrusion and even roasting [11]. In fact, aflatoxin B1 and B2 can resist destruction at temperatures as high as 289˚C [5].

Aflatoxins have many consequences for human health. For example, they are involved in hepatic carcinogenesis and have immunosuppressive properties that can affect fetuses [12]. Other consequences are dependent on the quantity, nature, and duration of exposure to aflatoxins [13]. At high doses, they can cause immediate death, however, they generally, cause a chronic diseases such as cancer, cirrhosis of the liver, and stunted-growth in children [14]. Furthermore, molds and aflatoxins have caused 1.2 billion dollars in economic waste [15] and quart of crops gathering had been lost, according to data from the Food and Agriculture Organization.

Due to the damage, they inflict on health and the economy, we seek to learn about and characterize the several molds and corresponding aflatoxins that are present in the different agroecological areas of Benin. To achieve this, the objective of this current work is to construct a map of the geographical distribution of *Aspergillus* section *Flavi* found in food and feed in Benin. Specifically, this work aims to determine the morphological characteristics and metabolic and molecular profiles of aflatoxins, which would ultimately be represented in an aflatoxin distribution map in Benin.

2. Material and Methods

2.1. Origin of Raw Materials

The samples comprised 318 strains of *Aspergillus* section *Flavi*. The presence of these strains was isolated from several food and feed sources in the eight agroecological areas of Benin. These strains were previously isolated during food contamination surveys performed in Benin by strains of *Aspergillus* section *Flavi*. The food sources were corn (*Zea mays*), popcorn, smoked and fermented fish, dry spices, *Manihot esculenta*, *Pennisetum glaucum*, *Sorghum bicolor*, peanut (*Arachis hypogaea*) and bambara groundnut. **Table A1** shows information regarding the origin of the strains.

2.2. Morphological Identification

Each strain was morphologically characterized on potato dextrose agar (PDA) and added to chloramphenicol. Macroscopic analysis was performed on malt extract agar (MEA) to isolate and characterize the strains of molds belonging to *Aspergillus* section *Flavi*. For microscopic analysis, an Olympus optic microscope, XSZ-107BN, No. 079931; at ×400, was used.

2.3. Molecular Identification

The DNA of the strain was extracted according the method of Aamir [16] with some modifications. Briefly, some quantity of mycelium was placed on the medium and crushed in a mortar with a lysis buffer (100 Mm Tris HCl; Ph = 8; 50 Mm EDTA; 3% SDS). Then, 1.5 mL was reground, placed in an Eppendorf tube and centrifuged at 12,000 rounds for 15 min. The supernatant was collected and mixed with 3 µl of RNase A (10 mg/ml), and incubated at 37˚C for 15 min. Then, an equal volume of phenol chloroform (50:50) was added and centrifuged at 12,000 rounds for 15 min. The chloroform phase described above was added to 1 mL of ethanol at 98˚ and incubated at −20˚C for 30 min.

DNA was quantified using a labelled Thermo Fisher Scientific spectrophotometer, model Evolution 60S, CAT 840-210100, SN 2R4326202, manufactured in China. Polymerization chain reaction (PCR) was used to amplify 1450 pb of the beta-tubulin sequence of the *Aspergillus* section *Flavi*s gene according to the Go Taq protocol. An Eppendorf thermal cycler was used for the PCR analysis. The total volume of the reactional mix was 50 µl. For the reaction mix reactional,

5 µl of Taq buffer (10×) was added to 5 µl of $MgCl_2$ (25 mM) with 5 µl of dNTP (2 mM) 1 µl of each primer (forward and reverse) (12.5 pM) and 1 µl of the DNA sample (10 ng). For RFLP, the PCR products were used with the BstYI restriction enzyme. The volume of the reaction mix was 20 µl. Then, 13 µl of the PCR product was added to 7 µl of the mix reaction mix from the total mix prepared as follows: 2 µl of BstYI, 1 µl of BstYI' buffer, and 62 µl of PCR water. The sample were incubated at 60˚C for 10 min and 15 µl of the RFLP product was loaded on a 1% agarose gel and viewed under 360 nm illumination.

2.4. Metabolites Characterization

The strains isolated and characterized on MEA were cultured on coconut cream agar medium to determine the production of aflatoxin under UV light and on *Aspergillus* flavus parasiticus agar to determine the production of aspergillic acid under sunlight by *Aspergillus* section *Flavi* after five days of incubation at 25˚C. Furthermore, secondary metabolites were extracted directly from the food and feed by the Bruce and *al*. method with some modifications. Secondary metabolites were also isolated from the strains cultured on PDA medium by the Azzoune [17] method. In this method, 5 mL of chloroform is added to a medium in which molds are grown and is vortexed for 2 min. The chloroform is 1.5 ml is placed in the Eppendorf tube and dried. Finally, 40 µl of methanol 90˚ added to the tube. The isolated mycotoxins were analyzed by chromatography plaque tests (10 cm × 10 cm). Chromatography plaques with aflatoxin standards (B1, B2, G1, G2) and samples were observed with 360 nm light.

3. Results

3.1. Morphological Characterisation

Based on the macroscopic characterization from Pitt and Hocking macroscopic characterization [18], all tested strains showed a color between yellow and green on the MEA medium, depending on the production of sclerotes such as *Aspergillus* section *Flavi* spp. (**Table A2**). Some strains showed a powdery form whereas others contained aerial mycelium. Several strains produced structures such as exudates and sclerotia. These observations are similar to those of Frisvad [19], who observed the same characteristics regarding *Aspergillus* section *Flavi* on MEA. This demonstrates that strains tested belonged to *Aspergillus* section *Flavi*. (**Figure 1** and **Figure 2**; **Table A3**).

The morphological analysis of secondary metabolites permitted the classification of *Aspergillus* section *Flavi* strains. Of these strains, 42% were identified as *A. parasiticus*, 21% as *A. flavus*, 19% as *A. coremiiformis*, and 7% as *A. tamarii*. It is important to remember that according to the morphological analysis, some strains contained two or three different *Flavi* subsections. It is because the strains numbers in the previous table are large over the initial strains numbers. In agroecological sites 1, 3, and 5, the strains were identified as mainly *A. nomius,* whereas in site 2, *A. flavus* was more present. In sites 5 and 8, *A. parasiticus* was

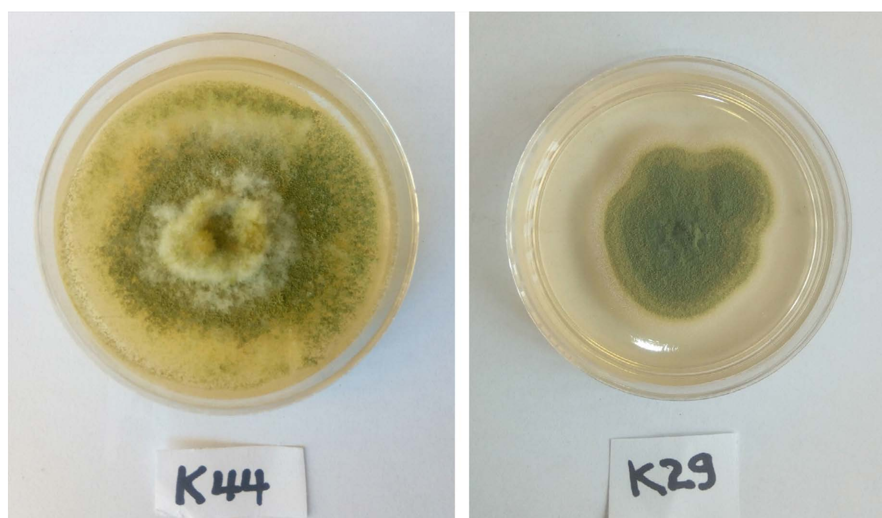

Figure 1. Visual aspect of two *Aspergillus* section *Flavi* strains on MEA J+7.

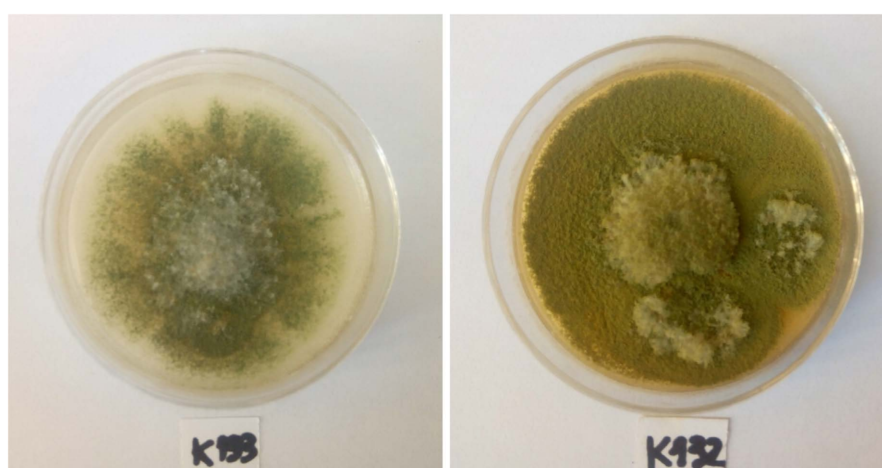

Figure 2. Visual aspect of two other *Aspergillus* section *Flavi* strains on MEA J+7.

abundant (**Table A4**).

3.2. PCR-RFLP

After beta-tubulin 2 gene amplification, 1450 pb of the DNA fragments were obtained (**Figure 3**). RFLP revealed two groups of the *Flavi* section, one group comprising three fragments of *A. flavus* at 336 pb, 438 pb, and 581 pb, and the other group comprising four fragments of *A. parasiticus* at 176 pb, 263 pb, 334 pb, and 576 pb (**Figure 4**).

3.3. Secondary Metabolites Characterization

3.3.1. Aspergillic Acid Identification

Of all the strains tested, 191 (60%) produced aspergillic acid: (**Table A5**).

3.3.2. Mycotoxins Analysis

Nearly half; of the A. section *Flavi* strains (41.82%) produced both aflatoxins B and G, whereas 28.93% produced only aflatoxins B. Furthermore, agroecological

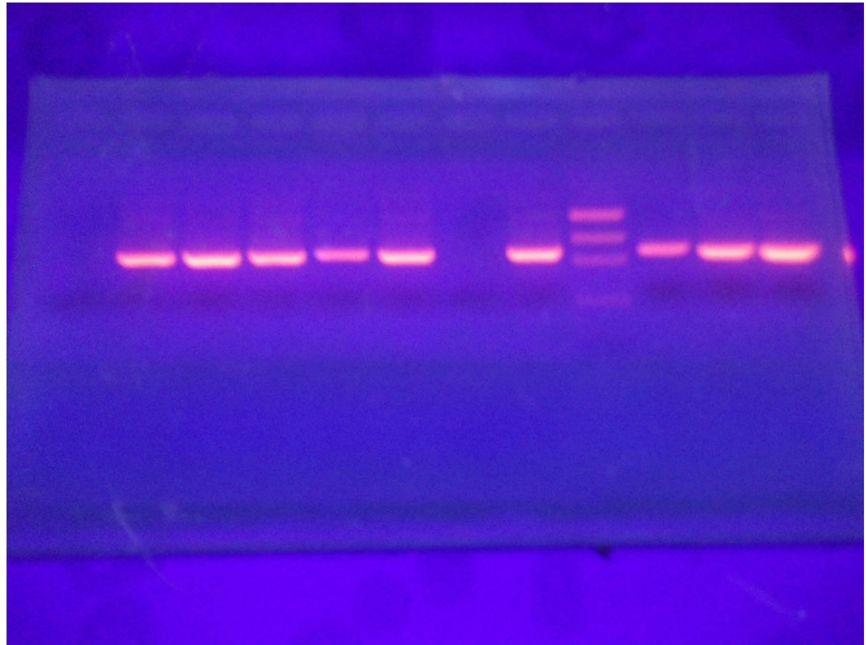

Figure 3. Agarose's gel (1%), result of beta tubulin 2 gene amplication by PCR migration.

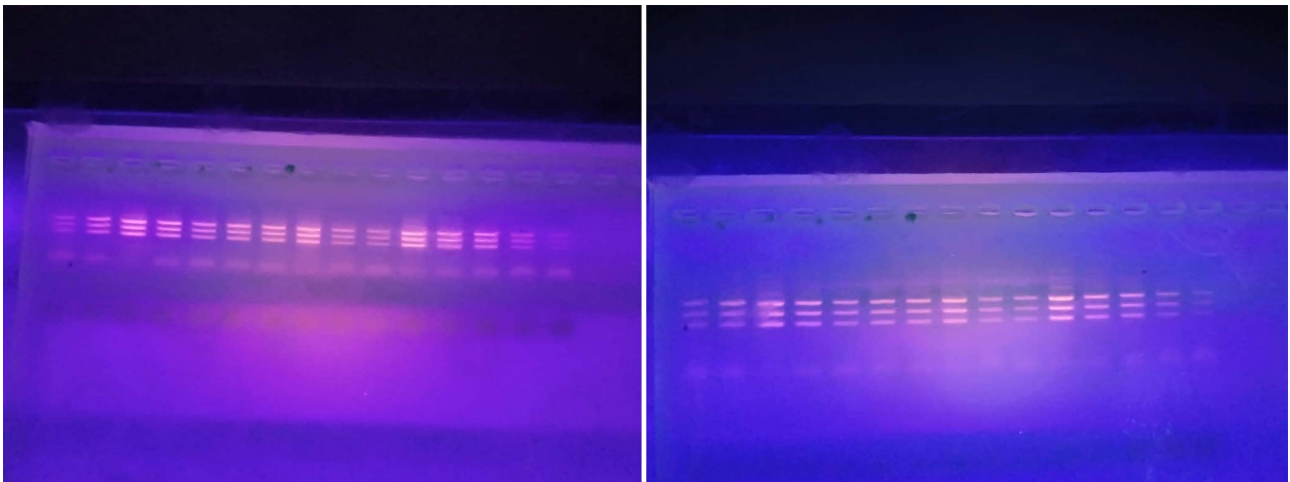

Figure 4. Agarose' gel (1%) result of RFLP migration (weight of DNA fragment: 1450 pb; weight of each DNA fragment: first line fragment from top: 576 pb; second line: 438 pb; last line: 336 pb).

sites 1, 3, and 4 were mainly contaminated by aflatoxins B, sites 5, 6, 7, and 8 contained both aflatoxins B and G, and in site 2, similar quantities of aflatoxins B and G were observed (Table 5A, Table 6A, and Table 7A).

4. Discussions

All food and feed samples were contaminated by several molds, mostly *Aspergillus-flavus*. Strains of *A. parasiticus*, *A. nomius*, *A. parvisclerotigenus*, *A. novoparasiticus*, *A. flavus,* and *A. togoensis* were identified from their morphological and metabolites characteristics. They presented a color between green and yellow on MEA after the seventh day of growth [19]. Similar results were found

by [20] and [21].

Indeed, Benin is a West African country located between 1˚ and 30˚N-S of latitude, with an average temperature varying between 26˚C and 32˚C [22]. These environmental conditions are ripe for molds development, most *Aspergillus* species grow primarily between 26˚C and 35˚C [23] with optimal growth occurring at a temperature of 33˚C [18]. The behavior of *A.* section *Flavi* in West Africa has been demonstrated in previous research. In addition, [24] showed the occurrence of *Aspergillus flavus* in peanut (*Arachis hypogaea*) in Burkina Faso. Furthermore, [25] demonstrated the same results as well as the existence of other molds such as A. *parasiticus* and *A. tamarii* in agroecological site 8.

The present survey demonstrated that food and feed from all 8-eight agroecological sites in Benin were contaminated by aflatoxins. Indeed, food and feed from the northern zones (1, 3, and 4) were shown to be strongly contaminated by aflatoxin B, while those from the southern zones (5, 6, 7, and 8) were contaminated by both aflatoxins B and G. The production of aflatoxins B is associated with the presence of *A. flavus* and *A. togoensis*, while both aflatoxins B and G are associated with the presence of *A. parasiticus*, *A. nomius*, *A. parvisclérotigenus*, and *A. novoparasiticus* in food and feedstuffs.

Generally, northern Benin is the primary agricultural zone. The majority of raw materials and staple foods are produced there. To increase production, farmers regularly use products such as fertilizer and; pesticides. Moreover, feedstuffs and staples are no longer kept in place. In addition, the northern zone is characterized by rough environmental conditions, demonstrating why a smaller population of *Aspergillus* and just one type of aflatoxins B were produced there. However, in the south, agricultural products are kept for a long time. In fact, southern Benin has the largest market in the country and even the West African zone. Indeed, agricultural products from several agroecological zones of the country and other countries such as Nigeria, and Togo are maintained in the international market of Dantokpa. The long duration of feedstuff and staple food storage facilitates the development and growth of many types of molds such as *Aspergillus*, and even the production of large quantities of the two types of aflatoxins (B and G). These results confirmed the previous survey regarding the link between the development of molds, aflatoxin production, and storage duration [26] [27]. The high production of aflatoxins in the south can be explained by the better environmental conditions for mold development, and environmental stresses such as drought, and temperature further aflatoxin production. In the south, as opposed to the North, there are four seasons that alternate throughout the year. The relative humidity of the environment encourages molds development and environment stress as the drought, temperature further aflatoxins production. In addition, the presence of aflatoxins B and G particularly in fish demonstrates cross-contamination among staples foods, as several types of fishes (dry, smoked and fermented) were generally sold with species that are strongly contaminated by *Aspergillus* and aflatoxins [28]. The transfer of

fungal strains was responsible for the production of aflatoxin in fish [25]. In particular, *A. parvisclerotigenus* and *A. novoparasiticus;* were identified in cassava in the transition zone between the north and south [29].

The map in (**Figure 5**) presents the distribution of *Aspergillus* section *Flavi* and their aflatoxins in Benin.

5. Conclusion

In summary, the present survey shows that staples foods, raw materials, and feedstuffs, including cooked food, are contaminated by aflatoxins in Benin. The north is contaminated by *A. flavus* and *A. togoensis,* producers of aflatoxins B (AFB1 and B2). The south, however, is mainly contaminated by *A. parasiticus* and *A. nomius,* producers of both aflatoxins B and G. Exposure to aflatoxins by

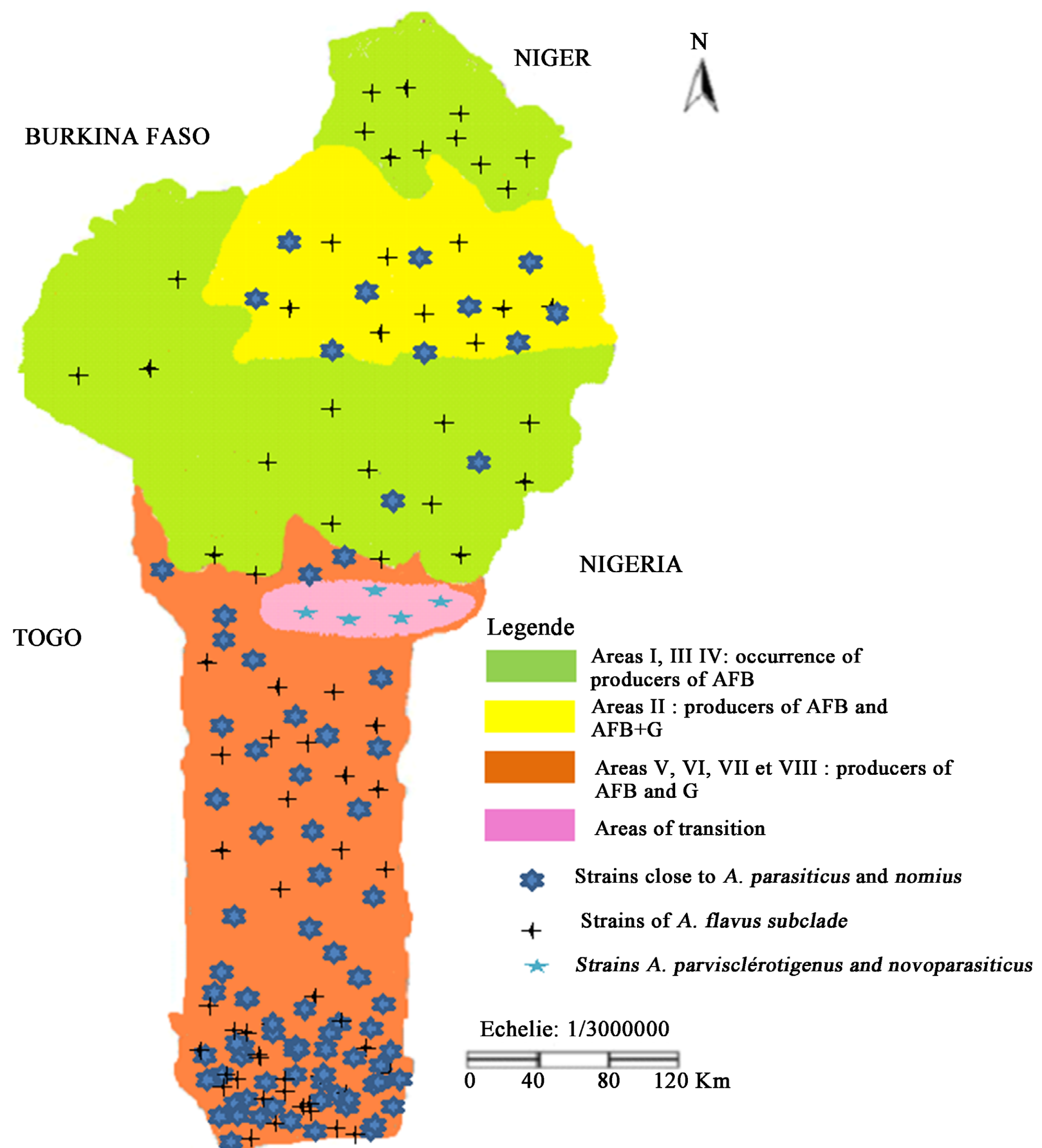

Figure 5. Repartition risk map linked of aflatoxins production in 8 agroecologic areas in Benin.

the population is inevitable owing to the consumption of contaminated food through mold development and the presence of mycotoxins. Further economic damage, which can increase poverty, molds contamination, and aflatoxins contamination, is very dangerous for population health. High exposure can lead to death. So, molds and aflatoxins food contamination could be avoided by observation of the good practices of harvest and good storage conditions.

Acknowledgments

Warm thanks to Dr. Yann ADJOVI who leds the present survey and supplied all reagents, dishes, culture mediums. We thank all members of the Laboratoire de Biochimie et de Biologie Moléculaire, which hosted the tests and manipulations.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ministère de l'Agriculture de l'élevage et de la pêche (2001).

[2] Jouany, J.P., Yiannikouris, A. and Bertin, G. (2009) Risk Assessment of Mycotoxins in Ruminants and Ruminant Products. In: Papachristou, T.G., Parissi, Z.M., Ben Salem, H. and Morand-Fehr, P., Eds., *Nutritional and Foraging Ecology of Sheep and Goats*, *Options Méditerranéennes*: *Série A. Séminaires Méditerranéens*, CIHEAM/FAO/NAGREF, Zaragoza, 205-224.

[3] Alonso, V.A., Pereyra, C.M., Keller, L.A.M., Dalcero, A.M., Rosa, C.A.R., Chiacchiera, S.M. and Cavaglieri, L.R. (2013) Fungi and Mycotoxins in Silage: An Overview. *Journal of Applied Microbiology*, **115**, 637-643. https://doi.org/10.1111/jam.12178

[4] Pinton, P. and Oswald, I.P. (2014) Effect of Deoxynivalenol and Other Type B Trichothecenes on the Intestine: A Review. *Toxins*, **6**, 1615-1643. https://doi.org/10.3390/toxins6051615

[5] El Khoury, R., Caceres, I., Puel, O., Bailly, S., Atoui, A., Oswald, I., El Khoury, A. and Bailly, J.-D. (2017) Identification of the Anti-Aflatoxinogenic Activity of *Micromeria graeca* and Elucidation of Its Molecular Mechanism in *Aspergillus flavus*. *Toxins*, **9**, Article No. 87. https://doi.org/10.3390/toxins9030087

[6] Smith, J.E. and Mos, M.O. (1984) Mycotoxins. Formation, Analysis and Significance. John Wiley & Sons, Chichester.

[7] World Health Organization (WHO) (2018) The Aflatoxins. 1-6.

[8] International Agency for Cancer Research (IACR) (2002) Monograph on Evaluation of Carcinogenic Risk to Humans, World Health Organization. Some Traditional Herbal Medicines, Some Mycotocins, Naphthalene and Styrene. Summary of Data Report and Evaluation, Vol. 82, IACR, Lyon, 171-175.

[9] Bailly, S., El Mahgubi, A., Carvajal-Campos, A., Lorber, S., Puel, O., Oswald, I.P., Bailly, J.-D. and Orlando, B. (2018) Occurrence and Identification of *Aspergillus* Section *Flavi* in the Context of the Emergence of Aflatoxins in French Maize. *Toxins*, **10**, Article No. 525. https://doi.org/10.3390/toxins10120525

[10] Giorni, P., Magan, N., Pietri, A., Bertuzzi, T. and Battilani, P. (2007) Studies on *Aspergillus* Section *Flavi* Isolated from Maize in Northern Italy. *International*

Journal of Food Microbiology, **113**, 330-338.
https://doi.org/10.1016/j.ijfoodmicro.2006.09.007

[11] Marin, S., Ramos, A.J., Cano-Sancho, G. and Sanchis, V. (2013) Mycotoxins: Occurrence, Toxicology, and Exposure Assessment. *Food and Chemical Toxicology*, **60**, 218-237. https://doi.org/10.1016/j.fct.2013.07.047

[12] World Health Organization (WHO) (2006) AFRO Food Safety Newsletter. Food Safety Unit (FOS).

[13] Bennett, J.W. and Klich, M. (2003) Mycotoxins. *Clinical Microbiology Reviews*, **16**, 497-516. https://doi.org/10.1128/CMR.16.3.497-516.2003

[14] Reddy, K.R.N., Reddy, C.S. and Muralidharan, K. (2009) Potential of Botanicals and Biocontrol Agents on Growth and Aflatoxin Production by *Aspergillus flavus* Infecting Rice Grains. *Food Control*, **20**, 173-178.
https://doi.org/10.1016/j.foodcont.2008.03.009

[15] Institut International d'Agriculture Tropicale (IITA) (2013).

[16] Aamir, S., Sutar, S., Singh, S.K. and Baghela, A. (2015) A Rapid and Efficient Method of Fungal Genomic DNA Extraction, Suitable for PCR Based Molecular Methods. *Plant Pathology & Quarantine*, **5**, 74-81.
https://doi.org/10.5943/ppq/5/2/6

[17] Azzoune, N. (2011) Etude des populations du genre Aspergillus et penicillium et de leurs mycotoxines isolées des épices et des légumes secs. Université M'hamed Bougara de Boumerdès, Boumerdes.

[18] Pitt, J.I. and Hocking, A.D. (2009) Fungi and Food Spoilage. 3rd Edition, Springer, London, 519.

[19] Frisvad, J.C., Hubka, V., Ezekiel, C.N., Hong, S.-B., Nováková, A., Chen, A.J., Arzankou, M., Larsen, T.O., Sklenář, F., Mahakarnchanakul, W., Samson, R.A. and Houbraken J. (2019) Taxonomy of *Aspergillus* Section *Flavi* and Their Production of Aflatoxins, Ochratoxins and Other Mycotoxins. *Studies in Mycology*, **93**, 1-63.
https://doi.org/10.1016/j.simyco.2018.06.001

[20] Nikiéma, P.A., Traoré, A.S. and Singh, B. (1995) Etude de la contamination de graines d'arachide par des aflatoxines produites au cours du stockage. *JARCB*, **1**, 2-16.

[21] Sanou, D. (2000) Etude de la prévalence des mycotoxines dans les produits agricoles du Burkina Faso: Cas de la contamination du maïs (*Zea mays* L.) par les aflatoxines et les fumonisines dans l'Ouest du Burkina. Université de Ouagadougou, Ouagadougou , 59.

[22] Agence Nationale de la Métérologie du Bénin (2019).
https://www.historique-meteo.net

[23] Klich, M.A. (2007) *Aspergillus flavus*: The Major Producer of Aflatoxin. *Molecular Plant Pathology*, **8**, 713-722. https://doi.org/10.1111/j.1364-3703.2007.00436.x

[24] Ouattara-Sourabie, P.B., Nikiema, P.A. and Traore, A.S. (2011) Caractérisation de souches d'*Aspergillus spp* isolées des graines d'arachides cultivées au Burkina Faso, Afrique de l'Ouest. *International Journal of Biological and Chemical Sciences*, **5**, 1232-1249. https://doi.org/10.4314/ijbcs.v5i3.72269

[25] Adjovi, Y.C.S., Tiko, G., Gnonlonfin, B.G. and Sanni, A. (2019) Morphologic and Molecular Characterization of *Aspergillus flavus* Isolated from Smoked, Fermented and Dried Fishes Sold in Main Markets of Cotonou (Benin). *Journal of Food & Industrial Microbiology*, **5**, Article No. 131.

[26] Borgemeister, C., Adda, C., Sétamou, M., Hell, K., Djomamou, B., Markham, R.H. and Cardwell, K.F. (1998) Timing of Harvest in Maize: Effects on Post Harvest Losses due to Insects and Fungi in Central Benin, with Particular Reference to *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). *Agriculture*, *Ecosystems and Environment*, **69**, 233-242. https://doi.org/10.1016/S0167-8809(98)00109-1

[27] Hell, K. and Mutegi, C. (2011) Aflatoxin Control and Prevention Strategies in Key Crops of Sub-Saharan Africa. *African Journal of Microbiology Research*, **5**, 459-466.

[28] Gnonlonfin, G.J.B., Adjovi, Y.C., Tokpo, A.F., Agbekponou, E.D., Ameyapoh, Y., de Souza, C., Brimer, L. and Sanni, A. (2013) Mycobiota and Identification of Aflatoxin Gene Cluster in Marketed Spices in West Africa. *Food Control*, **34**, 115-120. https://doi.org/10.1016/j.foodcont.2013.04.021

[29] Adjovi, Y.C.S., Bailly, S., Gnonlonfin, B.J.G., Tadrist, S., Querin, A., Sanni, A., Oswald, I.P., Puel, O. and Bailly, J.D. (2014) Analysis of the Contrast between Natural Occurrence of Toxigenic *Aspergilli* of the *Flavi* Section and Aflatoxin B1 in Cassava. *Food Microbilogy*, **38**, 151-159. https://doi.org/10.1016/j.fm.2013.08.005

Appendix

All Tables:
https://docs.google.com/document/d/1361T6YVrK92Tts_3nWWd_8t-e1PQrm_i/edit#heading=h.gjdgxs

Table A1. List of strains from *Aspergillus* section *Flavi* collected within agroecologic area in Benin.

https://docs.google.com/document/d/130Cn8CNW_Jhz_oM78XAH1mzOabusaKSPovdDCoKE2U8/edit

Table A2. References section *Flavi*'s clades.

https://docs.google.com/document/d/1TbER0QnrgYJVfYXQum-mBSbsKkOq1_lxr8nhmV-Nxgs/edit?usp=sharing

Table A3. Mascroscopic's characterization of strains.

https://docs.google.com/document/d/1GVvZ8wr4CAq4W2f7tUberr2TfDY09wDij4FCYq3WefU/edit?usp=sharing

Table A4. Global metabolic profile of strains.

https://docs.google.com/document/d/1-5s_Wl2aopaB0hRK8OKW3Iik_W3M6yNKMDIZhPB7owQ/edit?usp=sharing

Table A5. Morphological identification results.

https://docs.google.com/document/d/1xrY-wEI_nGz96rzZ6goeGk0ykW9lKiMNTl_nYAcxaPc/edit?usp=sharing

Table A6. Aflatoxins production distribution.

https://docs.google.com/document/d/1Vqtev8UgEj8IEVL_O5ZdTe9sTSpXhNsF-dG-gkgxwIk/edit?usp=sharing

Table A7. Synthesis of strains identification.

https://docs.google.com/document/d/1PZ6n8Mfxu566dLB1p3Aj9bJ6bTGry45NMFmtFsjaVaM/edit?usp=sharing