Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 16(1): 367-377, February 2022

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Characterization of strains of *Aspergillus flavus* and *A. parasiticus* isolated from groundnut (*Arachis hypogea*), rice (*Oryza sativa*) and maize (*Zea mays*) in Senegal

Abdoulaye FAYE[1*], Youssoupha DIOP[2], Diégane SARR[2], Papa Yoro GUEYE[2] and Atanasse COLY[3]

[1]Plant Protecton Directorate (DPV), Laboratory of Phytopathology; BP: 20054 Thiaroye, Senegal.
[2]Alioune Diop University of Bambey, UFR SATIC, EMEPA, Website: www.uadb.edu.sn, BP 30 Bambey, Senegal.
[3]Cheikh Anta Diop University of Dakar (UCAD)/Faculty of Science and Technology/Laboratory of Phytochemistry and Analysis (LPA), BP: 5005 Dakar-Fann, Senegal.
[*]Corresponding author; E-mail: blayefaye@yahoo.fr ; Tel: (+221)775055759

Received: 04-10-2021 Accepted: 03-02-2022 Published: 28-02-2022

ABSTRACT

The contamination of certain food crop with aflatoxin poses a real public health problem for consumers and causes many market losses for exporters. Thus, several research works are oriented in the direction of developing methods to combat aflatoxinogenic fungus. This study aimed at identifying and characterizing strains of *Aspergillus flavus* and *A. parasiticus* on groundnut, maize and rice seeds grown or imported into Senegal. Four species (*A. niger*, *A. tamarii*, *A. flavus* and *A. parasiticus*) were isolated from the seeds with incidences ranging from 0 to 100% depending on the samples and their provenance. Six strains of *A. flavus* and 3 strains of *A. parasiticus* have been identified and characterized on CYA and G25N culture media. The characterization focused on the morphological characteristics (color and appearance, mycelial growth) of the colonies on the different culture media, and on some microscopic characteristics such as the density of sporulation (production of conidia) and the appearance of the conidiophore. Strains of *A. flavus* were more frequent on groundnuts and isolates with the same traits were also identified from rice and maize samples, hence the 3 strains of *A. parasiticus* were isolated.

Keywords: *Aspergillus*, groundnut, maize, rice, Senegal.

INTRODUCTION

Cultivated plants are subject to several types of biotic stress that can lead to considerable production losses. The same applies to their post-harvest products whose warehouses are often subject to pest attacks, fungal infestations and/or parasitic infections that can cause significant damage to producers and in some cases health risks to consumers. Aflatoxins are toxic substances produced by certain types of cosmopolitan molds (fungi) that can contaminate food crops and pose a serious threat to humans and livestock. They are also a significant economic burden, causing 25% or more of the destruction of food crops worldwide each year (WHO, 2008). Two

DOI : https://dx.doi.org/10.4314/ijbcs.v16i1.31

closely related species of fungi are the main culprits in the production of aflatoxins: *Aspergilus flavus* and *A. parasiticus*. Under favorable conditions, which are usually found in tropical and subtropical regions, these molds can invade food crops before and after harvest. Pre-harvest aflatoxin contamination is quite limited for maize, cottonseed, groundnuts and nuts. After harvest, however, this contamination can affect various other crops such as coffee, rice and spices. Storage in inappropriate conditions that promote mold growth (warm and humid storage environments) can typically lead to very high levels of contamination (Baglo et al., 2019). Several types of aflatoxins (14 or more) are present in nature, but four (aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2)) are particularly dangerous for humans and animals because they are found in all major food crops. Nevertheless, human exposure comes mainly from contaminated nuts, seeds or their derivatives. In addition, aflatoxin M1 (AFM1), a metabolizing product of AFB1, can also be found in milk in areas of high aflatoxin exposure. Subsequently, humans may be exposed to this aflatoxin through the consumption of milk and dairy products, including breast milk, especially in areas where animals are fed with poor quality seeds (WHO, 2008). Indeed, several studies have shown that aflatoxins are potentially carcinogenic and can affect all organ systems, especially the liver and kidneys. AFB1, for example, is known to be carcinogenic in humans, with its ability to cause liver cancer being significantly increased in the presence of hepatitis B virus (HBV) infection.

Thus, the need to overcome the contamination of foodstuffs by aflatoxins implies that of developing methods for the identification of aflatoxinogenic strains and making effective strategies to combat the development of these fungi. In this vein, it was subject in this study to characterize *A. flavus* and *A. parasiticus* strains isolated from groundnut, rice and maize samples collected in Senegal.

MATERIALS AND METHODS

Plant material

The plant material (Table 1) consists of 33 samples including 8 of maize (7 collected in Senegal in the localities of Marina Ndiathbé, Aéré Lao and Djilor Saloum and 1 sample imported from Argentina), 13 of rice (including 3 from China, Thailand and India and 10 locals from Richard Toll, Madina Ndiathbé, Aéré Lao and Adéane) and 12 of groundnuts taken from Kaolack, Diourbel and Ngoundiane areas.

Methods

Sampling

Samples were taken by purchase from informal trade. In each locality, a composite sample of 5 Kg of rice, groundnut and/or maize was collected from 5 randomly selected vendors at the rate of 1 Kg purchased from each seller. The different samples were placed in sachets bearing the respective labels (place and date of collection, sample code).

Incubation, transplanting and purification

In the laboratory, a subsample of 75 seeds was randomly collected from each sample. They were soaked with 75% alcohol for 1 minute and then incubated in wet chambers (petri dishes of 90 mm in diameter lined with blotter paper wet with sterilized distilled water) at the rate of 15 seeds per box with 5 repetitions. From 24 hours of incubation, the young mycelial colonies appeared on the seeds were taken and transferred to the Mazé culture medium (composed of a liter of white bean broth added 20 g of glucose, 2 g of bacteriological peptone and 20 g of agar-agar). Subsequently, they were successively transplanted on the non-selective medium of Czapek Yeast Extract Agar (CYA) consisting of K2HPO4 (1 g), concentrated Czapek (10 ml), metal solution (1 ml), yeast extract (5 g), sucrose (30 g), agar-agar (15 g), distilled water (1 L), until pure strains were obtained at 25°C. Through microscopic observations, all *Aspergillus* species were identified and strains with a hyaline and non-partitioned conidiophore were retained as belonging to *A. flavus* or *A. parasiticus*.

Identification and characterization of the strains

The AFPA medium (bacteriological peptone (10 g), yeast extract (20 g), ferric ammonium citrate (0.5 g), chloramphenicol (100 mg), agar-agar (15 g), dichloran (1 ml to 0.2% in ethanol), distilled water (1 L), pH 6.5) was used to identify *Aspergillus flavus* and *A. parasiticus* according to the protocols of Pitt et al. (1983) and Cotty (1994), while the characterization of the strains was carried out on Glycerol Nitrate Agar (G25N) medium. *Aspergillus* strains were identified by observation of crop characteristics during 7 days of incubation: growth rate (colony diameter), colony colour and texture (Christensen, 1981; Hocking, 1982; Doster et al., 2009). In addition to the crop characteristics, samples from the colonies were observed under an optical microscope on a millimeter object slide to assess the density of conidia (spores) and describe the appearance of the conidiophore.

Statistical analyses

The quantitative data collected on this study were entered on the Excel software, which also made it possible to express them graphically. They were subjected to statistical analyses using Costat software version 7.2. An analysis of variance and a comparison of the means were made on the values recorded on the diameter of the mycelial colonies of the strains and their conidia number per mm^2, using the Student-Newman-Keuls test at the 5% threshold.

Table 1: Plant material composition.

Number	Sample code	Crop	Origin
1	Md1m	maize	Madina Ndiathbé
2	Md2m		Madina Ndiathbé
3	Al1m		Aéré Lao
4	Al2m		Aéré Lao
5	Ds1		Djilor Saloum
6	Ds2		Djilor Saloum
7	Ds3		Djilor Saloum
8	Arg		Argentine
9	Rc1	rice	Richard Toll
10	Rc2		Richard Toll
11	Rc3		Richard Toll
12	Md1		Madina Ndiathbé
13	Md2		Madina Ndiathbé
14	Al1r		Aéré Lao
15	Al2r		Aéré Lao
16	Ad1		Adéane
17	Ad2		Adéane
18	Ad3		Adéane

19	Th		Thaïlande
20	Ch		Chine
21	In		Inde
22	Kl1	groundnut	Kaolack
23	Kl2		Kaolack
24	Kl3		Kaolack
25	Kl4		Kaolack
26	Dl1		Diourbel
27	Dl2		Diourbel
28	Dl3		Diourbel
29	Dl4		Diourbel
30	Ng1		Ngoundiane
31	Ng2		Ngoundiane
32	Ng3		Ngoundiane
33	Ng4		Ngoundiane

RESULTS

Specific diversity and frequency of *Aspergillus* on seeds

Four species of *Aspergillus* (*A. flavus*, *A. parasiticus*, *A. niger* and *A. tamarii*) were identified from incubated groundnut, rice and maize seeds with varying frequency and incidence depending on crops and the provenance of the samples. *Aspergillus flavus* was found on almost all samples of maize, groundnuts and rice, but with a greater incidence on groundnut seeds. Indeed, the average incidence peak (69.33%) was recorded on the Dl2 sample from Diourbel (Figure 2). *Aspergillus parasiticus* was identified on 75% of maize samples and 84.6% of rice samples, alever with a relatively low average incidence on grains (Figures 1 and 2). Indeed, the highest average incidence of *A. parasiticus* (7%) was recorded on the RC1 rice sample from Richard Toll (Figure 2). In addition, it was found that none of the peanut samples showed an infestation by *A. parasiticus*. The species *A. niger* was present on 87.5% of maize samples, 92.3% of rice and 66.6% of groundnuts with a peak incidence of 100% recorded on the Kl1 sample taken from Kaolack (Figure 3). However, it was noted that no peanut samples from Ngoundiane were infested with *A. Niger*. Concerning the species *A. tamarii*, it was found on 62.5% of maize, 30.7% of rice and 91.6% of groundnut samples, with a peak incidence of 8.33% observed on the Dl3 peanut sample taken from Diourbel (Figure 3).

Characterization of the *A. flavus* and *A. parasiticus* strains

Six (06) strains of *Aspergillus flavus* and three (03) of *Aspergillus parasiticus* were identified and characterized (Table 3) on CYA and G25N media after 7 days of culture at 25°C. Statistical analyses revealed a significant difference in colony diameter ($p = 0.0013$) and number of conidia ($p = 0.0008$) between the different strains.

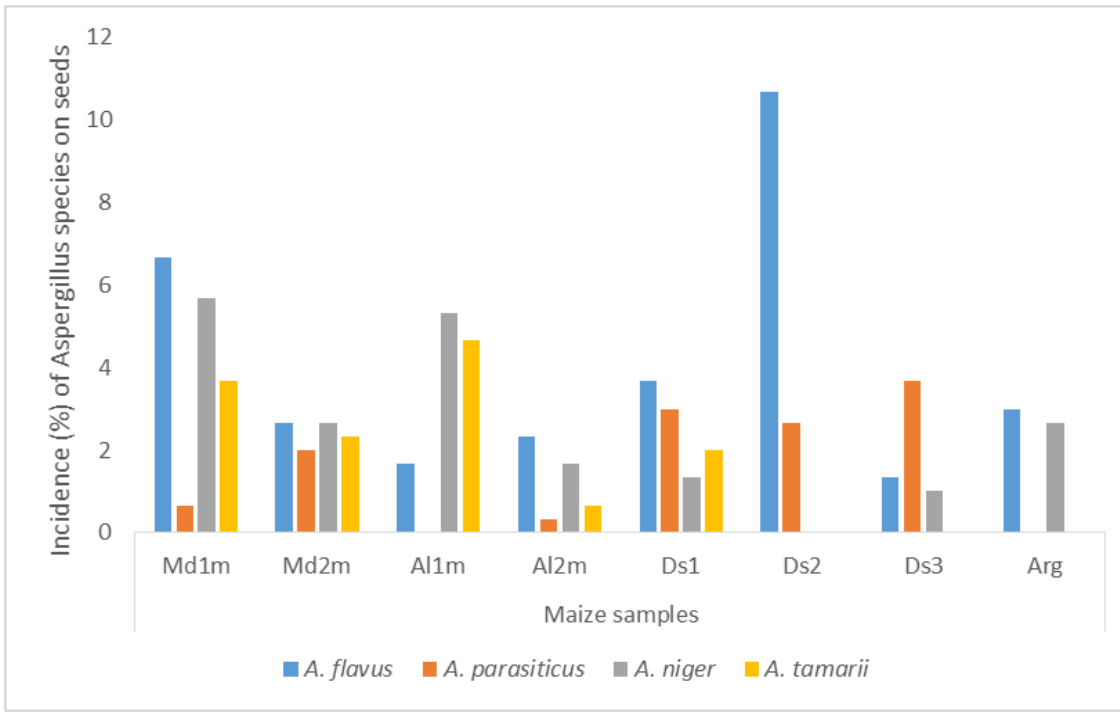


Figure 1: Variation of the incidence of seeds infestation by different *Aspergillus* species in the maize samples.

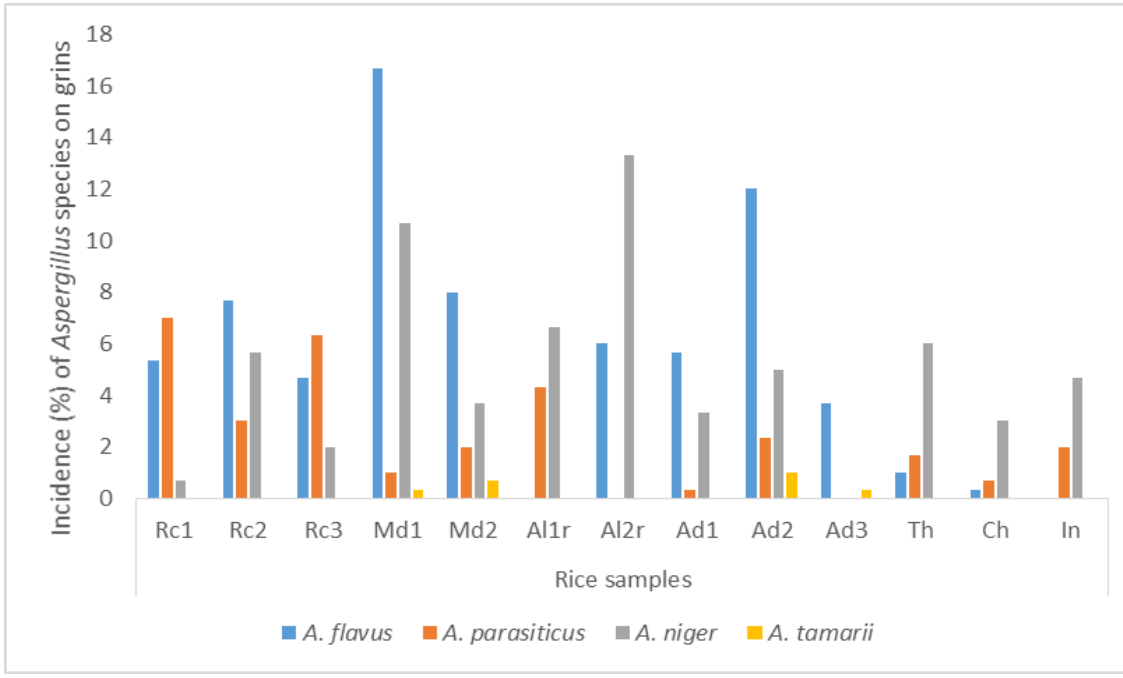


Figure 2: Variation of the incidence of grins' infestation by different *Aspergillus* species in the rice samples.

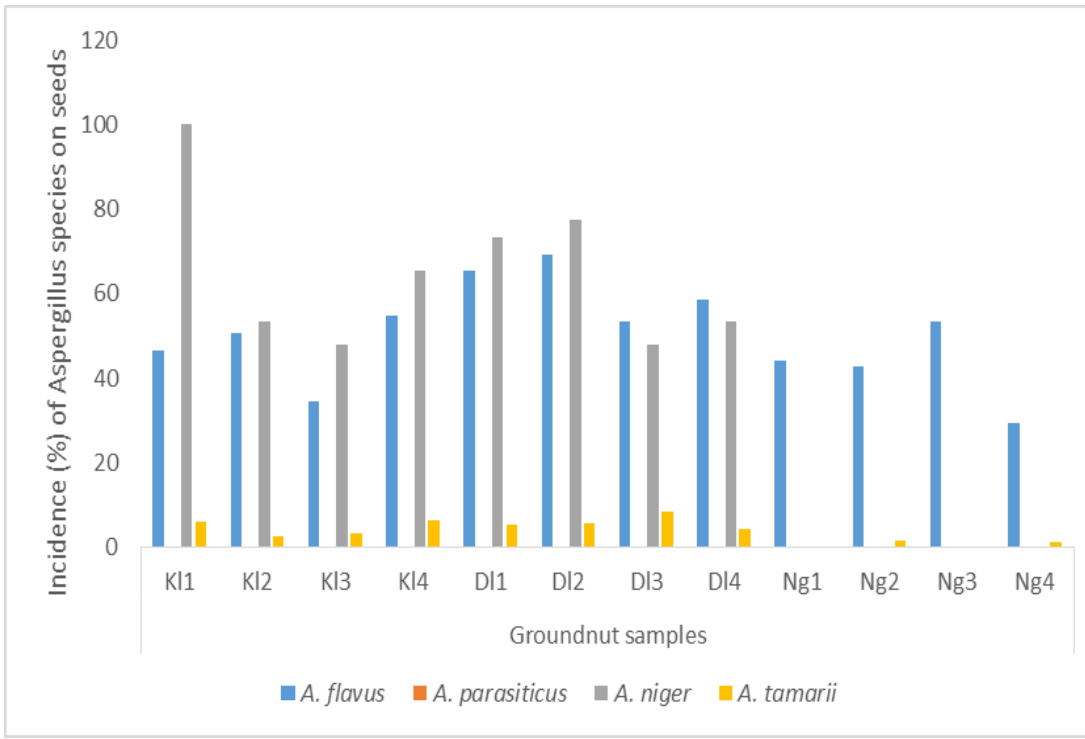


Figure 3: Variation of the incidence of seeds infestation by different *Aspergillus* species in the groundnut samples.

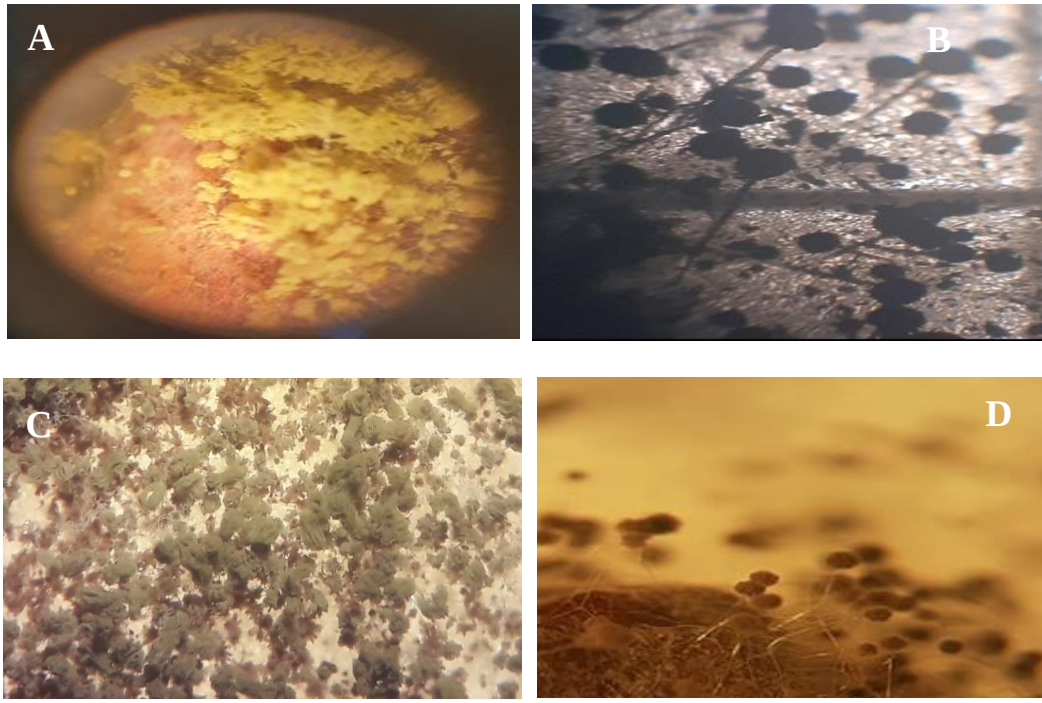
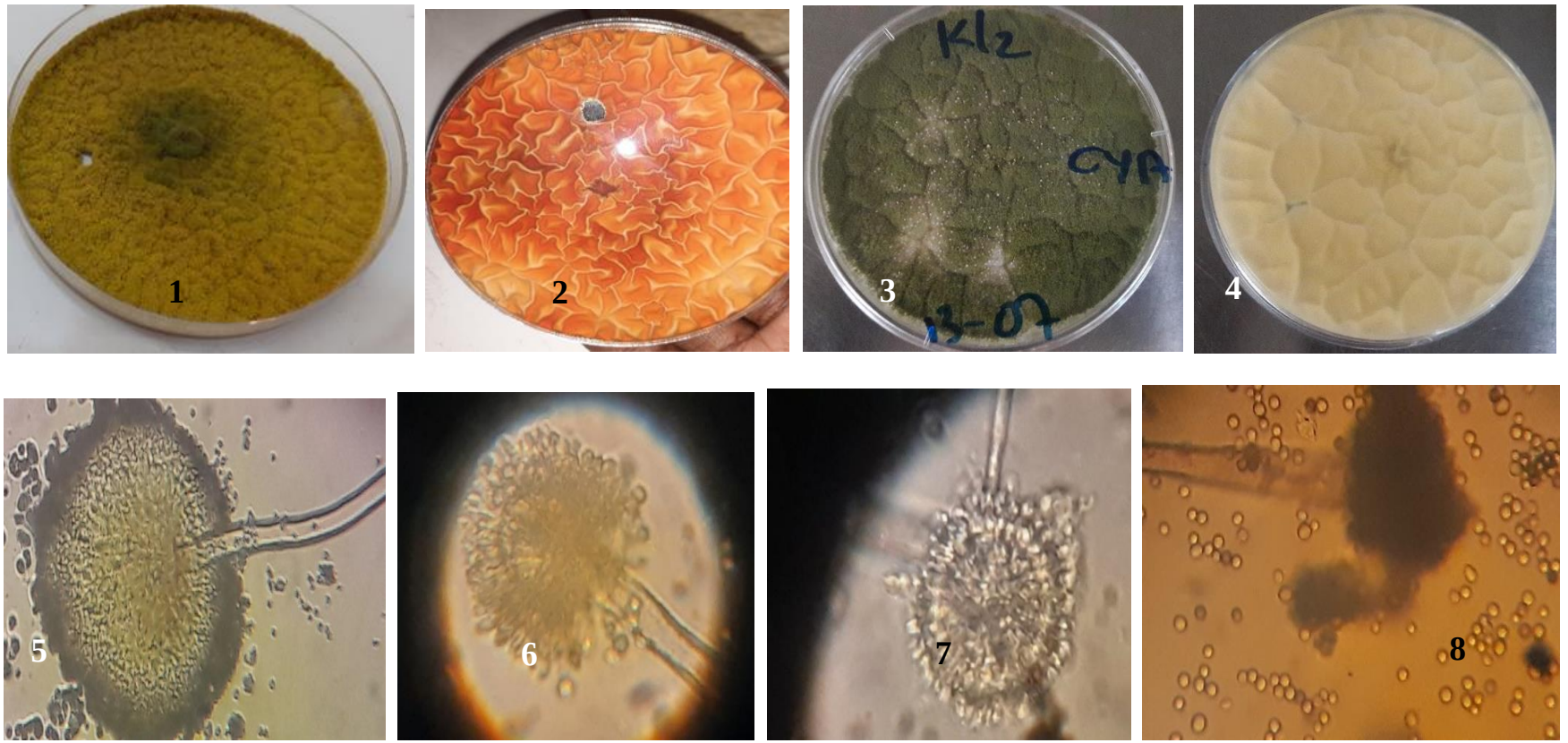


Photo 1: Illustrations of the different Aspergillus species on infested seeds.
A: *A. flavus* on groundnut ; B : *A. niger* on rice grin
C : *A. parasiticus* on maize ; D : *A. tamarii* on maize.

Table 3: Macroscopic and microscopic characteristics of the *Aspergillus* strains on the 7th day of incubation on CYA and G25N culture media at 25°C.

Species	Strains	Characteristics	
		on CYA	on G25N
A. flavus	Ng2	Light green colony with white outline, reverse yellowish brown and radiant. Biserized conidian head. Average colony diameter 7.9 cm. 390 spores average per mm^2	Light green colony pulling to yellow with a whitish outline. Reverse yellow-brown. Average colony diameter 6.4 cm
	Dl1	Homogeneous olive-green colony with a velvety appearance, reverse orange yellow with a wrinkled appearance. Average colony diameter 8.8 cm. 375.5 spores average per mm^2	All green colony with an irregular outline. Reverse orange-yellow with a radiant appearance. Average colony diameter 7.3 cm
	Dl2	Greenish-yellow colony without scleroties, reverse orange-yellow with a wrinkled appearance. Biserized conidian head. Average colony diameter 7.8 cm. 333.25 spores average per mm^2	Homogeneous green colony without scleroties with a whitish border, reverse orange-yellow. Average colony diameter 4.9 cm
	Kl1	Yellowish-green colony with white scleroties on the surface, dense structure, reverse orange-yellow. Average colony diameter 8 cm. 425 spores average per mm^2	Olive-green colony with a few white scleroties on the surface, radiant velvety texture at the edge, denser and greyish in the center. Average colony diameter 6.1 cm
	Kl2	Green colony with lots of scleroties, white outline, reverse light-yellow with a partitioned appearance. Average colony diameter 8.5 cm. 363 spores average per mm^2	Olive-green colony with lots of scleroties. Fluffy structure. Reverse yellow-brown. Colony diameter 7.6 cm
	Kl3	Homogeneous green colony, reverse dark yellow pulling to gray. Average colony diameter 9 cm. 486.75 spores average per mm^2	Dark greenish colony without scleroties with a yellowish-white outline. Yellowish reverse with a radiant appearance. Colony diameter 5.5 cm
A. parasiticus	Rc1	Colony dark green with white outline, reverse yellow-orange. Uniserized Conidian head. Average colony diameter 9 cm. 416.5 spores average per mm^2	Dark green colony with yellowish-white outline, reverse dark orange. Average colony diameter 7.1 cm
	In	Dark green colony with some scleroties, yellow side. Uniserized Conidian head. Average colony diameter 7.9 cm. 234 spores average per mm^2	Dark green colony with yellowish-white outline, reverse orange-yellow. Average colony diameter 6.7 cm
	Ch	Colony dark green with without scleroties, reverse orange. Uniserized Conidian head. Average colony diameter 8.3 cm. 326.25 spores average per mm^2	Dark green colony with yellowish-white outline, reverse orange-yellow. Average colony diameter 6.1 cm


1
2
Kl2
CYA
3
4
5
6
7
8

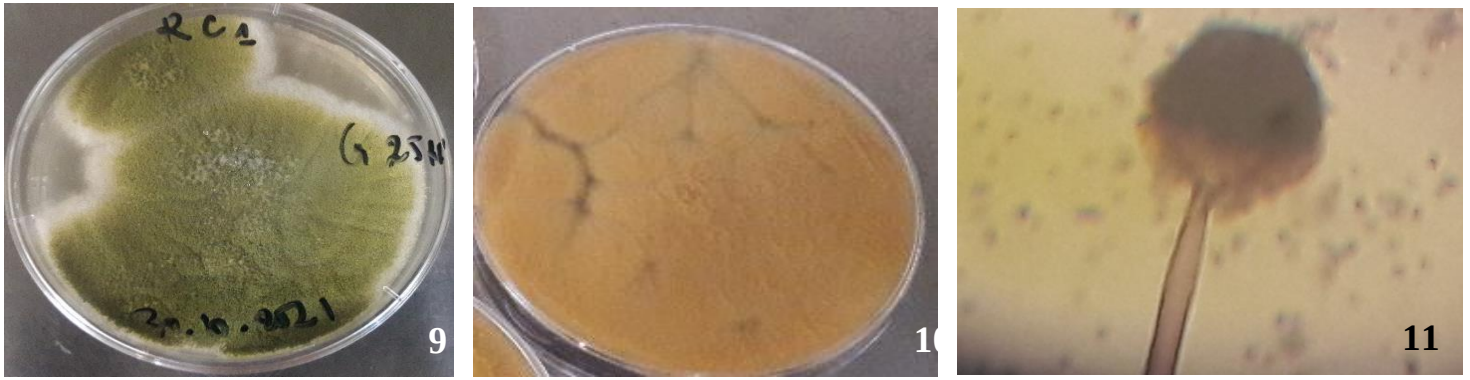


Photo 2: Illustrations of cultural and microscopic characters of some of the A. flavus and A. parasiticus isolated strains.

1 and 2: face and reverse of the Dl2 strain colony on CYA; 3 and 4: face and reverse of the Kl2 strain colony on CYA; 5: Conidian head of the Ng2 strain (GX40); 6: conidian head of the Dl1 strain (GX40); 7: conidian head of the Kl3 strain; 8: conidiophore and conidia of the Ds1 strain (GX40); 9 and 10: face and reverse of the Rc1 strain colony on CYA and G25N; 11: Conidiophore of the Rc1 strain (GX40)

DISCUSSION

Our results on infestation levels of groundnut, rice and maize samples taken from different collection sites in Senegal show a high frequency of *Aspergillus* species in the seeds. Among them, the strains of *A. flavus* occupy a very important place with a peak of average incidence that could reach 69.33% on peanut seeds in the sample taken from Diourbel. *A. parasiticus* was more common on rice and maize samples, with relatively lower incidences. The incidences of *A. niger* and *A. tamarii* also vary depending on the speculation and its origin. Indeed, *Aspergillus* are ubiquitous saprophytic fungi that grow easily in tropical areas where temperatures generally vary between 25 and 45°C. The uneven incidences recorded in seeds from the different collection sites could be explained by an unequal distribution of inocula of different *Aspergillus* species in soils, but also by post-harvest seed storage conditions. According to Barros et al. (2005), *Aspergillus* are microscopic fungi that contaminate crops in the fields or during storage in silos or granaries. And, when climatic conditions are favourable, some strains of the genus *Aspergillus* produce aflatoxins which are secondary metabolites known to be carcinogenic, immunosuppressive and teratogenic (WHO, 2006). In particular, the species *Aspergillus flavus* and *A. parasiticus* are the best known and have been the subject of several studies that have demonstrated their ability to produce aflatoxins (Ito et al., 2001; Johnsson et al., 2008; Doster et al., 2009; Reddy et al., 2009; Ouattara-Sourabie et al., 2011).

Macroscopic and microscopic observations of *A. flavus* and *A. parasiticus* cultured on CYA and G25N culture media have noticed differences in their cultural characteristics, sporulation density and mycelial growth. These differences are explained by morphological characteristics specific to each strain, but also by the difference in the composition of the culture media. Indeed, the presence of glycerol and nitrate in G25N and that of metal solution and sucrose in CYA are at the origin of the differences in coloration, sporulation density and mycelial growth observed in isolated strains. In our experimental conditions, we were able to see that all strains of *A. flavus* and *A. parasiticus* showed faster mycelial growth on the CYA medium than on G25N. This observation is consistent with those of Ouattara-Sourabie et al. (2011) who state that the *A. flavus* is more stable in the CYA environment than on the G25N.

The presence of *A. flavus* and/or *A. paraiticus* in foods does not necessarily induce the production of aflatoxins. Indeed, their synthesis depends on environmental conditions (temperature and relative humidity), the nature of the substrate, the stage of development of fungi and their ability to produce or not aflatoxins. Thus, even if these fungi are able to grow at temperatures between 10 and 45°C (Diener and David, 1986), the synthesis of aflatoxins occurs only at temperatures between 20 and 35°C; when the relative humidity is between 80 and 85%. These atmospheric conditions are very frequently encountered in Senegal. Therefore, it is easy to understand that plant products, especially peanuts and maize, are in these areas the preferred substrates of aflatoxiinogen fungi, especially at the time of harvest, when the pods and seeds contain 30 to 35% water.

Conclusion

This study made it possible to make a contribution to the identification of *Aspergillus* species infesting 3 plant foods (peanuts, maize, rice) in Senegal and to the characterization of the strains of the *flavi* section potentially aflatoxinogens. The results obtained showed incidences ranging from 0 to 100% of the species *A. niger*, *A. tamarii*, *A. flavus* and *A. parasiticus* depending on the samples studied and their origin. In addition, 6 strains of *A. flavus* and 3 strains of *A. parasiticus* could be

distinguished and characterized according to their macroscopic characteristics on the culture media CYA and G25N and some of their microscopic traits. For the purpose of a more complete characterization of strains, it seems essential to consider a study of their suitability for the production of aflatoxin.

ACKNOWLEDGMENTS

The authors thank the Senegalese Institute of Agricultural Research (ISRA) through the Center for the Development of Horticulture for its collaboration in this research. They also thank the Chemical Industries of Senegal (ICS) for the financial support granted.

REFERENCES

Baglo DE, Faye A, Fall M. 2019. Determination of aflatoxin in maize produced in two regions of Togo. *Adv Food Technol Nutr Sci Open J.*, **6**(1): 42-46. DOI: 10.17140/AFTNSOJ-6-167

Barros D, Torres A, Chulze S. 2005. *Aspergillus flavus* population isolated from soil of Argentina's peanut-growing region. Sclerotia production and toxigenic profile. *J. Sci. Food. Agric*., **85**: 2349– 2353. DOI: 10.1002/jsfa.2257

Christensen M. 1981. A synoptic Key and evaluation of species in the *Aspergillus flavus* group. *Mycol.*, **73**: 1056-1084. DOI: 10.1080/00275514.1981.12021441

Cotty PJ. 1994. Comparison of four media for the isolation of *Aspergillus flavus* group fungi. *Mycopathol.*, **125**: 157-162.

Doster MA, Cotty PJ, Michailides TJ. 2009. Description of a Distinctive Aflatoxin-Producing Strain of *Aspergillus nomius* that Produces Submerged Sclerotia. *Mycopathol.*, **168**: 193-201.

Hocking AD. 1982. Aflatoxigenic fungi and their detection. *Food Technol. Aust.*, **34**: 1- 3.

Ito Y, Peterson SW, Wicklow DT, Goto T. 2001. *Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section. *Flavi. Mycol. Res.*, **105**: 233239.

Johnsson P, Lindblad M, Thim AM, Jonsson N, Vargas EA, Medeiros NL, Brabet C, Quaresma de Araújo M, Olsen M. 2008. Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts. *World Mycot. J.*, **1**(2): 127-137.

Ouattara-Sourabie PB, Nikiema PA et Traore AS, 2011. Caractérisation de souches d'*Aspergillus* spp isolées des graines d'arachides cultivées au Burkina Faso, Afrique de l'Ouest. *Int. J. Biol. Chem. Sci.*, **5**(3): 1232-1249.

Pitt JI, Hocking AD, Glenn DR. 1983. An improved medium for detection of *Aspergillus flavus* and *A. parasiticus*. *J. Appl. Bacteriol.*, **54**: 109-114.

Reddy KRN, Saritha P, Reddy CS, Muralidharan K, 2009. Aflatoxin B1 producing potential of *Aspergillus flavus* strains isolated from stored rice grains. *Afric. J. Biotechnol.*, **8**(14): 3303-3308.

WHO. 2008. La charge mondiale de morbidité : mise à jour de **2004**. World Health Organization. Disponible sur http://www.who.int/evidence/bod