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Occurrence of Aflatoxins in Rice Intended for Infant Flour Production in Ouagadougou, Burkina Faso

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Authors' contributions

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

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ABSTRACT

A total of four samples of rice intended for infant flour production in Ouagadougou were received at the Physico-chemistry laboratory of Food Technology Department (DTA) for quality control. The latter were also tested for *Aspergillus* section *Flavi* presence and analyzed for aflatoxins B1, B2, G1 and G2 content using high performance liquid chromatography (HPLC). Among the twenty (20) strains of mold isolated from these samples, three *Aspergillus* section *Flavi* were obtained and cultivated in "*Aspergillus flavus* and *parasiticus* Agar (AFPA)" to ascertain if they belong to *Aspergillus flavus* or *Aspergillus parasiticus* species. The qualitative ability of aflatoxin production was also performed by fluorescence emission under ultra violet light at 365 nm after four days of incubation at 30 °C on Coconut Agar Medium (CAM). Statistical analysis results showed that 75% of samples were contaminated with total aflatoxins (AFs) with contents ranging from 0.54 ± 0.06 to

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2.40 ± 0.07 µg/Kg. Aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2) were detected in two contaminated samples. AFB1 had the highest concentration as compared with other aflatoxins. A significant level of contamination ($p < 0.0001$) was observed in sample R441 compared to other sample types.

Keywords: Aflatoxins; Rice; identification; Aspergillus; Burkina Faso.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most consumed cereal staple foods worldwide [1]. Worldwide contribution of rice is about 20% of total consumed staple foods and 40% of energy intake [2]. Rice occupies the 4th place among the cereals cultivated in Burkina Faso, both in terms of area, production and annual consumption. In fact, the annual consumption of rice in Burkina Faso greatly exceeds 400,000 T and is increasing at a rate of about 5.6%. It is the cereal with the most yield (2.18 T/h) ahead of the three other crops, namely sorghum, millet and maize [3]. Rice plays an important role in the economy and in the consumption of urban and rural households in West African countries. In addition, because of the ease and low costs of its preparation, the low prices and the regularity of its supply, rice has become a staple foodstuff and a strategic product for a good part of the world population [4]. However, the development of the rice value chain comes up against multiple obstacles, including the quality of the grain due to the different conditions of production, harvesting, drying, threshing and storage, which constitute an obstacle for this sector [5].

In Burkina Faso, rice is grown in wet soils for almost the entire year, we have upland rice as well as that of the lowlands and slopes of certain rivers [4]. This permanent humidity coupled with poor rice harvesting and threshing practices make the panicles more subject to invasion by toxigenic molds [6]. In fact, in most areas with high production, mature grains are cut and left on the ground while farmers take care of other crops (peanuts, cowpeas, etc.). These practices leave cereals within the reach of insects, rodents and soil microorganisms such as toxigenic molds [7]. Fungi infestation in the grain occurs in the field, during its formation, or in the warehouse, if this latter is not adequately treated during storage. Reddy and Sathyanarayana [8] listed 143 different fungi species that were found in rice. But the isolates majorly described as mycotoxigenic belong to the genera *Aspergillus*, *Penicillium* and *Fusarium*. Aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2), citrinin (CIT), sterigmatocystin (STE), fumonisins B1 (FB1), B2 (FB2) and B3 (FB3), zearalenone (ZEA), cyclopiazonic acid (CPA), patulin (PAT), gliotoxin (GLI) and different trichothecenes are some of the mycotoxins isolated from rice. Aflatoxins (AFs) are the more frequently met and were produced by *Aspergillus* section *Flavi* such as *Aspergillus flavus* which produces only aflatoxins B, *Aspergillus parasiticus* which produces both B and G aflatoxins, *A. nomius*, *A. bombycis*, *A. pseudotamari*, and *A. ochraceoroseus* [9]. Among aflatoxins, AFB1 is the most toxic form for mammals and presents hepatotoxic properties [10]. However, mycotoxin producing fungi is less commonly reported for rice than for many other cereal crops [11] but rice represents a very good substrate for fungal growth and toxinogenesis since it is used as an ideal culture medium to test the toxigenic potential of isolated strains. As a result, these foods are subject to export restrictions to countries with regulations on mycotoxin residue limits. In fact, the maximum residue limit fixed by the European Union for AFB1 in rice and its derivate products is 2 µg/kg, except for baby foods and processed cereal-based foods for infants and young children, where the Commission established a level of 0.1 µg/kg [12]. For other aflatoxins, a limit of 4 µg/kg has been set [13]. The *Codex Alimentarius* is in force in several countries in West Africa and stipulates that the acceptable limits of the AFB1 and AFs content in rice grain are respectively 2 µg/Kg and 5 µg/Kg [13]. Thus, our study aimed to determine the occurrence of all kinds of aflatoxins in rice samples intended for infant flour production in Ouagadougou using different methods and to identify the molds responsible for aflatoxinogenesis by morphological and physiological criteria.

2. MATERIALS AND METHODS

2.1 Determination of pH and Moisture Content

The moisture content was measured by drying 5 g of rice samples in an oven at 105 °C ± 2 °C during 12 h [14]. For sample pH determination, standards methods were used. The tests were repeated three times.

2.2 Sampling and Fungi Isolation from Rice Sample

Four local samples rice intended for infant flour production in Ouagadougou city of Burkina Faso were received in January 2021 at the Physico-chemistry laboratory of Food Technology Department (DTA) for quality control. Molds were isolated according to Ulster or direct method. It involved depositing directly several rice grains on Potato Dextrose Agar (PDA) at 30 °C until four days. A total of twenty (20) fungi were collected from the local rice samples. *Aspergillus spp* strains among these isolates has shown powdery, filamentous appearance, yellowish, greenish, or blackish colonies which may be the interest strains. Also, a microscopic view of isolates conidiophores of strains was performed on an optical microscope to put away strains having a septate and non-hyaline conidiophore. Thus, among these isolates, three (3) related to *Aspergillus spp*. were selected for morphological and physiological characterization. "*Aspergillus flavus* and *parasiticus* Agar (AFPA)" was used to identify *Aspergillus flavus* and *Aspergillus parasiticus* according to Pitt et al. [15] and Cotty [16] protocols. Systematic determination and the morphological identification of the strain were made on the Potato Dextrose Agar (PDA) at 25 °C and 37 °C depending on the methods used by Christensen [17] and Hocking [18]. Inoculation was done in three points equidistant.

2.3 Reference Strains

Three reference strains served as a basis for comparison. It was UBOCC-A-106031 (*Aspergillus flavus* aflatoxinogenic) of French origin, UBOCC-A-111042 (*Aspergillus parasiticus* var. globosus aflatoxinogenic) of Japanese origin as well as S_2 (*Aspergillus flavus* aflatoxinogenic) strain previously isolated from groundnut in Burkina Faso and identified [19]. These reference strains stored at - 4 °C were Revivified in sabouraud broth at 30 °C for 4 days. They were then subcultured onto the Potato Dextrose Agar (PDA) medium and incubated at 30 °C for four days, for a comparison of cultural and microscopic characters with strains isolated from rice grain.

2.4 Aflatoxins Analysis

2.4.1 Qualitative analysis

The qualitative analysis of aflatoxin production by three *Aspergillus* section *Flavi* was carried out by means of a visual assessment of a blue fluorescence emitted by the strains on the Coconut Agar Medium (CAM) at 30 °C. Thus, the isolated strains and those of references have all been cultured on this medium at 30 °C for four days. Thus, 20 g of shredded coconuts have been homogenized for 5 min with 300 ml distilled water heated to about 70 °C. The solution was filtered through tissue. The agar has been added to coconut broth at the rate of 20 g/l then the mixture was autoclaved at 121 °C for 15 min. The final pH was adjusted to 7.0. UV plate was used to view colonies under ultra violet light at 365 nm. When the test is negative there is no blue fluorescence around the colonies observed [20].

2.4.2 Quantitative analysis by HPLC

Aflatoxins quantitative analysis of the four rice samples was carried out by HPLC at the toxicology department of National Public Health Laboratory (LNSP) in Ouagadougou. The principle consisted in extracting the aflatoxin in the samples of rice grains by the use of suitable organic solvents, in purifying this aflatoxin on an immuno-affinity column and then in identifying and quantifying it [14].

Extraction: 25 g of ground rice was weighed and placed in a jar. 125 ml (V_1) of extraction solution (70% methanol / 30% distilled water) was added to the sample and stirred for 20 min. The solution was then filtered with pleated filter paper and 15 ml of the filtrate (V_2) were taken by means of a pipette and transferred in a conical flask then completed with 30 ml of distilled and homogenized water to give a total volume of 45 ml (V_3).

Purification: It was done using immunoaffinity columns. They were placed on the variant cuvette and using a pipette, 15 ml of the mixture (filtrate + distilled water) (V_4) was taken and deposited in the solvent reservoir of the immunoaffinity column where it will retain the desired aflatoxin molecules. The molecules were then washed with 20 ml of distilled water. 1.5 ml (V_5) of methanol was put in the immunoaffinity column to elute the aflatoxin molecules and this solution was collected in vials and sent to HPLC for detection and quantification of aflatoxins molecules.

Identification and quantification: This step of the analysis was based primarily on HPLC. 50 µL (V_6) of standard solution was injected into the injection loop. Detection and quantification were

carried out through a chromatogram. The aflatoxins are eluted in the following order G2, G1, B2, B1, with respective retention times of approximately 6 min, 8 min, 9 min and 11 min. Each aflatoxin peak in the chromatogram from the analysis of the test sample was identified, by comparing the retention times to those of the corresponding reference standards. The quantitative determination was carried out according to the external standard method with integration of the peak area or the height of the peak, then comparison with the corresponding value of the standard substance.

Calculation of results: The mass m_t in grams of the test sample present in the fraction of the filtrate taken from the immunoaffinity column (V_4) was calculated using the following equation:

$$m_t = m_0 \frac{V_2 \times V_4}{V_1 \times V_3}$$

- m_0 is the mass of the test sample in grams (m_0 = 25 g)
- V_1 is the total volume of the first mixture, in milliliters (V_1 = 125 ml)
- V_2 is the volume of the fraction of the first filtrate taken for dilution, in milliliters (V_2 = 15 ml)
- V_3 is the total volume of the diluted solution, in milliliters (V_3 = 45 ml)
- V_4 is the volume withdrawn from the diluted solution, in milliliters (V4 = 15 ml).

The mass fraction of each aflatoxin, w_i in micrograms per sample kilogram was calculated, using the following equation:

$$\boldsymbol{w_i = \frac{V_5 \times m_i}{V_6 \times m_t}}$$

- V_5 is the volume of the eluate, in microliters (V_5 = 1500 µl)
- V_6 is the volume of the sample extract purified and injected, in microliters (V_6 = 50 µl)
- m_i is the mass of each aflatoxin *i* present in the injection volume corresponding to the measured peak area or to the peak height recorded on the calibration curve, in nanograms
- m_t is the mass of the test sample present in V_4 taken for the immunoaffinity column, in grams.

The mass fraction of total aflatoxins will be obtained by adding the mass fractions of the four aflatoxins.

2.5 Statistical Analysis

The differences in aflatoxins concentration and those of physicochemical analyzes in rice samples were compared by Analysis of variance (ANOVA) using XLSTAT-Pro 7.5.2 software. Interpretation of values was performed using Newman-Keuls test at probability level p = 5%. The results were expressed as mean ± SD and the measures were repeated three times (n=3).

3. RESULTS

3.1 Determination of pH and Moisture Content

The moisture content of rice samples ranged from 8.65 to 8.92%, indicating that our samples are slightly damp (Table 1).

Table 1. Moisture rate of rice samples

Sample	Moisture rate (%)
R435	8.70 ± 0.07[a]
R436	8.65 ± 0.04[a]
R439	8.84 ± 0.04[b]
R441	8.92 ± 0.01[b]

Values denoted by different superscripts in each column are not significantly different at 5% probability level according to Newman – Keuls test.significance level< 0.0001

The pH results of the various rice samples analyzed indicate that all the samples are slightly acidic, with pH values ranging from 5.03 to 5.05 (Table 2).

Table 2. pH values of rice samples

Sample	pH
R435	5.05 ± 0.01[a]
R436	5.04 ± 0.03[a]
R439	5.03 ± 0.01[a]
R441	5.03 ± 0.01[a]

Values denoted by different superscripts in each column are not significantly different at 5% probability level according to Newman – Keuls test. significance level < 0.0001

3.2 Morphological Identification of Mold Isolates

A total of twenty (20) mold strains were isolated from the four rice samples, of which three (3) belong to *Aspergillus* section *Flavi* (Table 3).

3.2.1 Macroscopic characteristics

Isolates F_1 and F_2 showed almost the same visual characteristics as those of UBOCC-A-111042 *A. parasiticus* reference strain on the PDA medium. Both isolates showed diameter between 30 to 35 mm, the colonies were deep yellow green, velutinous in its center compared to that of reference S_2-*A. flavus* and UBOCC-A-106031 *A. flavus*, yellow-brown reverse with increased pigmentation. However, UBOCC-A-111042 *A. parasiticus* were green with white mycelia and roughened with age after splitting into columns and produced white sclerotia in the center. Isolate F_1 produced exudates. As for F_3 isolate, it was rather similar to the strain S_2 *A. flavus* and UBOCC-A-106031 *A. flavus* reference. The strain F_3 isolate also exhibited the same visual characteristics as both reference strains UBOCC-A-106031 and S_2-*A. flavus* on the PDA medium and with, however, F_3 presents yellow green colonies with white mycelia at the edge and produced exudates (Table 4 and Fig. 1).

3.2.2 Microscopic characteristics

In optical microscopy, all strains have a conidiophore, not septate, hyaline with a radiant conidial head and variations at the seriation level, form and dimensions of the vesicle, hyphae and conidia (Table 3 and Fig. 2). Thus, reference strain UBOCC-A-111042 *A. parasiticus* and both local isolates F_1 and F_2 predominantly were uniseriate with radiate conidia heads, of which some were knodding at the tip of the stipe. The vesicle was pyriform to globose and measured 24-35 µm in diameter and is yellow for the local strain F_1 and the reference strain UBOCC-A-111042 *A. parasiticus*. Stipe length was 320-480 µm for F_1 and 380-450 µm for F_2 with thick and smooth walls which were uncolored. Conidia size ranged between 4-6 µm; smooth; globose and green in color. Reference strains UBOCC-A-106031 *A. flavus* and isolate F3 showed a longer rough conidiophore (400-930 µm) than reference strain UBOCC-A-111042, which had conidial heads tilted to the side end of the stipe. Both have biseriate vesicles which were spherical to globose with a diameter between 14-43 µm. The conidia size ranged between 3-5 µm; globose; smooth to finely rough and yellow-green color.

3.3 Aflatoxins Production Capacity

Analysis of strains ability to produce aflatoxins through blue fluorescence emission from colonies in Coconut Agar Medium (CAM) showed all local isolates F_1, F_2 and F_3 and those of reference strains UBOCC-A-106031, UBOCC-A-111042 and S_2 are aflatoxins producer. These results were corroborated with their positive response on AFPA medium (Table 6).

3.4 Aflatoxins level in Rice Grains

The aflatoxin level in rice samples intended for infant flour production from Ouagadougou city are recorded in Table 7 and Fig. 5. AFs ranged from 0.54 ± 0.06 to 2.40 ± 0.07 µg/Kg. The highest AFB1 contents was 2.18 ± 0.04 µg/Kg. 75% of the samples were found to be contaminated with AFs. These were R436, R439 and R441. R441 had the highest concentration (2.40 ± 0.07 µg/Kg), R436 had the lowest concentration (0.54 ± 0.06 µg/Kg), while R435 was free from aflatoxins.

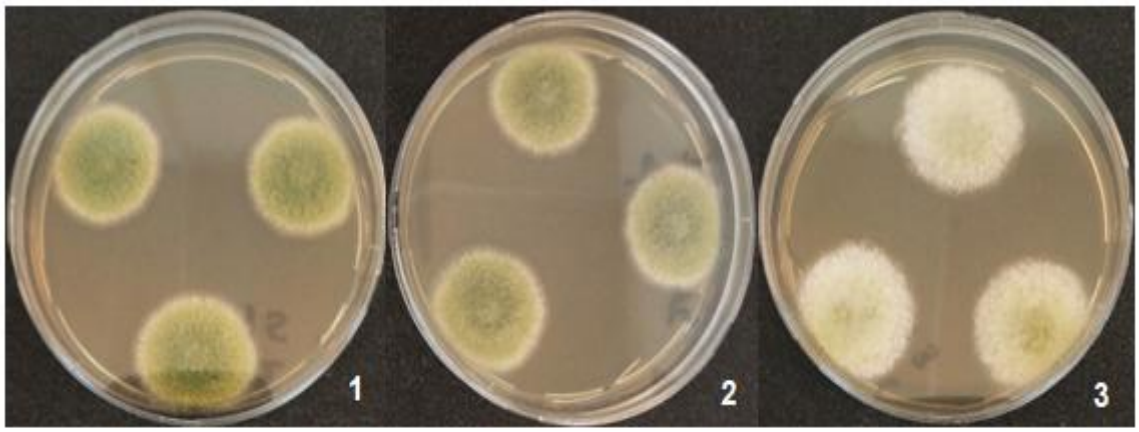

Fig. 1. Strains macroscopic aspects F_1 (1), F_2 (2), UBOCC-A-111042 (3), in PDA medium at 4 days of incubation at 30 °C

Table 3. Number of *Aspergillus* section *Flavi* isolates from collected rice samples

Sample	Number of total molds isolates	Number of *Aspergillus* section *Flavi* isolates	Isolates code
R435	3	0	-
R436	5	1	F_1
R439	4	1	F_2
R441	8	1	F_3
Total	20	3	-

Table 4. Macroscopic characters on the 4th day of incubation at 30 °C of *Aspergillus* section *Flavi* strains on PDA medium

Strain	Macroscopic characteristics
F_1	Colonies were deep yellow green, velutinous in its center compared to that of S_2-*A. flavus* and UBOCC-A-106031 *A. flavus*, yellow-brown reverse with increased pigmentation, some white sclerotia were in the colonies center whose diameter was about 30–35 mm, produced exudates.
F_2	Colonies were deep yellow green, velutinous in its center compared to that of S_2-*A. flavus* and UBOCC-A-106031 *A. flavus*, yellow-brown reverse with increased pigmentation, some white sclerotia were in the colonies center whose diameter was about 30–35 mm.
UBOCC-A-111042 *A. parasiticus var. globosus* aflatoxinogenic	Colonies were green with white mycelia and roughened with age after splitting into columns and produced white sclerotia in the center in the colonies whose diameter was about 30–35 mm.
F_3	Colonies were white, becoming yellowish yellow green then dark green, downy, raised; the texture is velvety radiant at the edge, fluffy, cottony and denser and dark green in the center, colonies smaller than those of S_2 whose diameter between 25-30 mm, produced exudates.
S_2 *A. flavus* aflatoxinogenic	Colonies were white, becoming yellowish green then dark green, downy, raised; the texture is velvety radiant at the edge, fluffy, cottony and denser and dark green in the center with white sclerotia, colonies diameter is between 40-55 mm with a yellowish reverse.
UBOCC-A-106031 *A. flavus* aflatoxinogenic	Colonies were white, becoming yellowish green then dark green, downy, raised; the texture is velvety radiant at the edge, fluffy, cottony and denser and dark green in the center, colonies smaller than those of S_2 whose diameter between 25-30 mm.

Table 5. Microscopic characters of *Aspergillus* section *Flavi* strains

Strain	Stipe texture	Seriation	Length Conidiophore (µm)	Vesicle diameter (µm)	Conidia size (µm)	Conidia Head/Shape
F_1	smooth	uniseriate	320-480	24-32	4-6	Radiate
F_2	smooth	uniseriate	380-450	29-35	4-6	Radiate
UBOCC-A-11042	smooth	uniseriate	200-430	28-32	4-7	Radiate
F_3	rough	biseriate	400-930	14-43	3-5	Radiate
S_2 *A. flavus*	rough	biseriate	400-800	14-43	3-5	Radiate
UBOCC-A-106031	rough	biseriate	450-930	14-43	3-5	Radiate

Table 6. Summary table of results of culture on AFPA and CAM

Strain	Orange yellow color in the colonies reverse on AFPA as shown Fig. 4	Aflatoxins production capacity by fluorescence emission under UV on CAM as shown Fig. 3
F_1	+	+
F_2	+	+
UBOCC-A-111042 *A. parasiticus*	+	+
F_3	+	+
S_2 *A. flavus*	+	+
UBOCC-A-106031 *A. flavus*	+	+

Table 7. Aflatoxin level in rice samples

Aflatoxin (µg/kg)	R435	R436	R439	R441	*Codex* limit
B1 (ppb)	nd	Nd	0.79 ± 0.03^b	2.18 ± 0.04^a	2
B2 (ppb)	nd	Nd	0.06 ± 0.01^b	0.22 ± 0.04^a	-
G1 (ppb)	nd	Nd	0.17 ± 0.03^a	nd	-
G2 (ppb)	nd	0.54 ± 0.06^a	nd	nd	-
significance level	< 0.0001				
AFs	nd	0.54 ± 0.06	1,01 ± 0.06	2.40 ± 0.07a	5

nd = not detected. For each line and for each rice sample, the means which are in common the same letter are not significantly different according to the Newman-keuls test at the 5% probability level

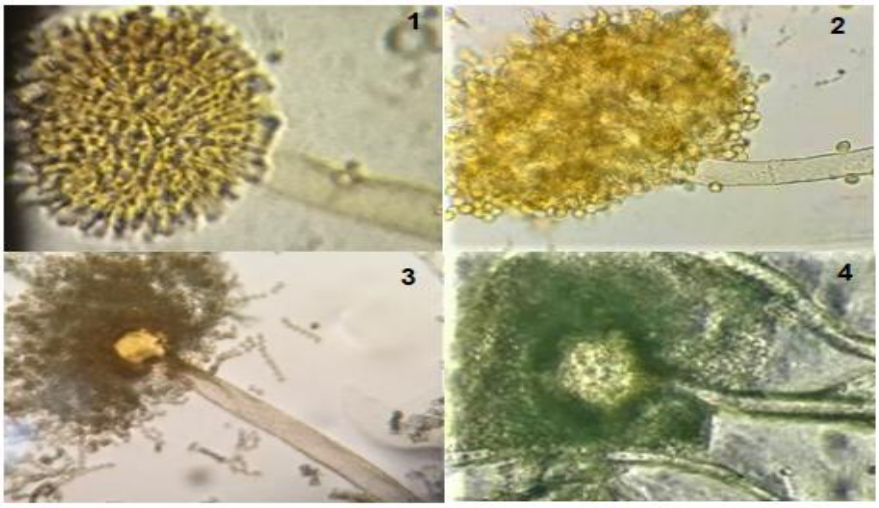

Fig. 2. Strains aspergillaires heads F_1 (1), UBOCC-A-111042 (2), F_3 (3) and UBOCC-A-106031 (4)

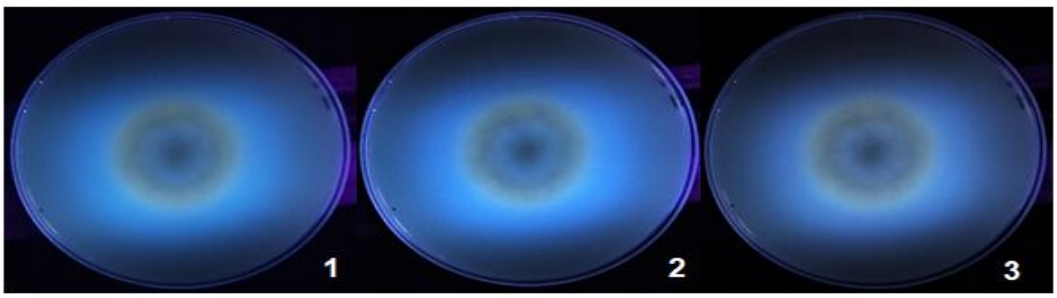

Fig. 3. Showed the detection aflatoxigenic *Aspergillus* section *Flavi* strain by Coconut Agar Medium (CAM) under UV light at 365 nm on the 4th day of incubation at 30 °C. (1) control of aflatoxigenic strain UBOCC-A-111042, (2) F_1 isolate and (3) F_3 isolate showing a blue-green fluorescent ring around the colony

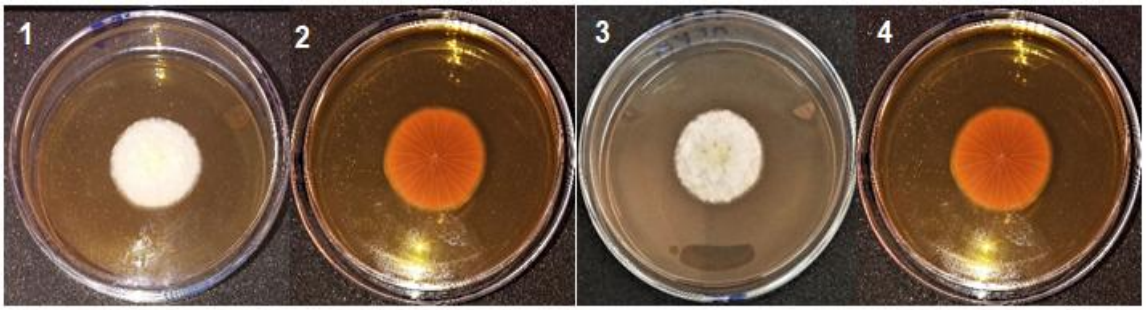

Fig. 4. Positives strains F_1 (1,2) and UBOCC-A-111042 (3,4) as (yellow-orange reverse) on AFPA medium after 4 days of incubation at 30 °C

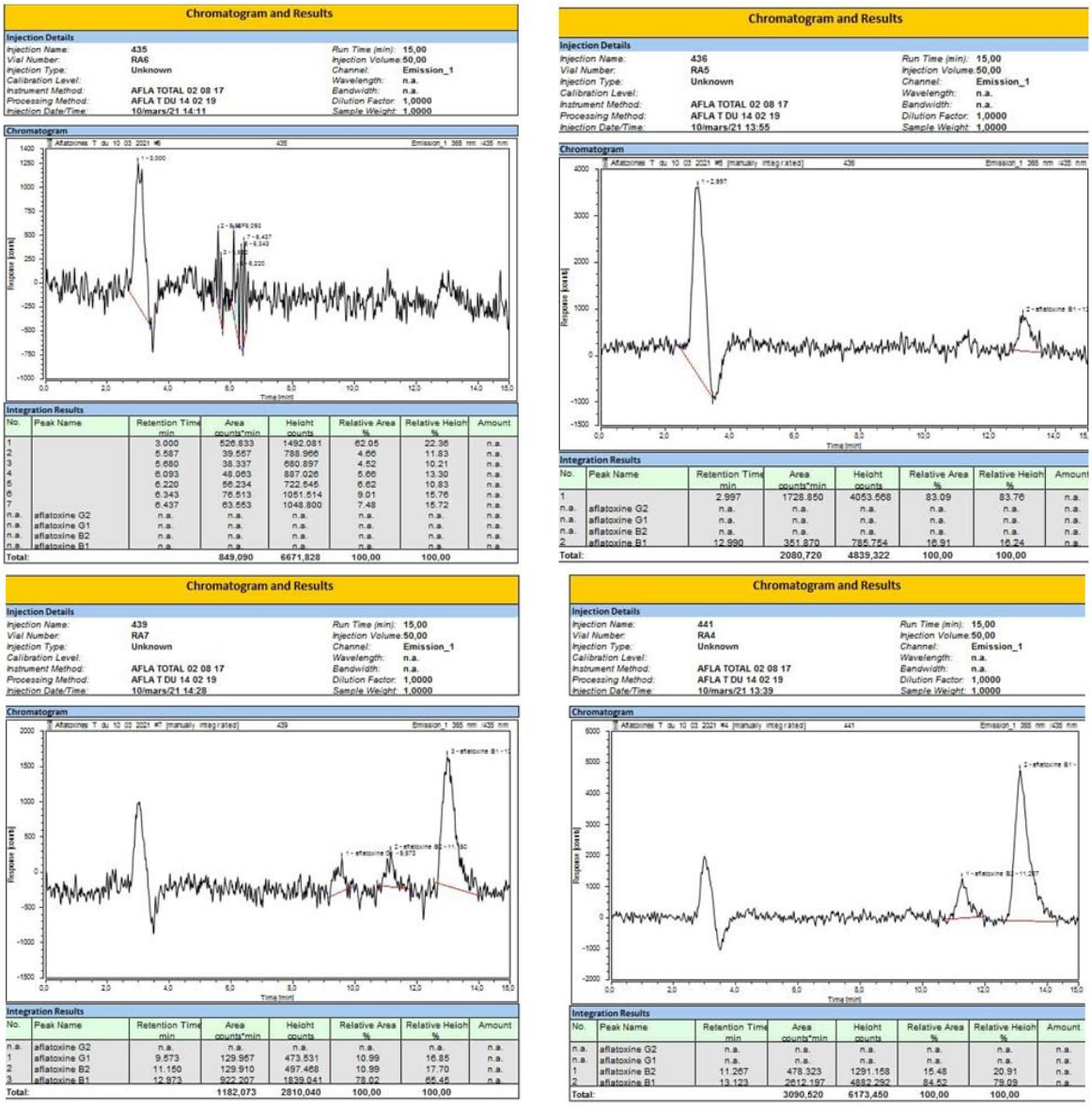

Fig. 5. HPLC chromatograms; lake of Aflatoxin in sample R435, AFG2 in sample R436, AFB1, AFB2 and AFG1 in sample R439, AFB1 and AFB2 in sample R441

4. DISCUSSION

Mycotoxins are among the most important concerns for food safety and human health, especially aflatoxins in agricultural products such as cereals. The results of the physicochemical tests obtained from the present study have revealed the character slightly acidic of the rice samples. These results were in concordance with those obtained from rice by Nguyen [21] in Vietnam. The pH values are very favorable to the growth of molds because according to Gauthier [22], molds can grow in a pH range from 3 to 8, with optimal growth being rather between 5 and 6. The production of mycotoxins takes place for pH close to optimal growth pH [23,24]. Thus, due to their acidity, many foods are much more subject to fungal than bacterial spoilage [25]. These results were in concordance with those obtained from the maize seed by Gao et al. [26] and Nyongesa et al. [27] respectively in northeast China and Kenya. In our study, moisture content in rice varied between 8.65 to 8.92%. According to Huong et al. [2] *Aspergillus flavus* shows optimal production of aflatoxins at 28 °C and 8-12% moisture content. Our data could be explained mold proliferation in rice sample. Indeed, the good drying of cereals is a determining factor of long-term conservation. Poorly dried rice increases the rate of grain breakage during threshing. The high moisture of the grains also facilitates the attack of the surface of the grains thus facilitating the access of the molds to the internal structures of the cereals which contain the nutrients [28]. For the different samples, statistical analyzes showed no significant differences between pH values and the different moisture contents.

Identification based on morphological and physiological characteristics revealed that isolates F_1 and F_2 of *Aspergillus* section *Flavi* have characteristics common to the reference strain UBOCC-A-111042 *A. parasiticus*. Therefore, they were classified as belonging to this species. This classification was based on the determination of the cultural characters observed on the PDA, and AFPA media and the microscopic characters which correspond to those of *A. parasiticus*. In addition, the analysis of aflatoxins in the rice samples R436 and R439 in which we isolated F_1 and F_2 showed the presence of AFB and AFG. According to some authors *A. parasiticus* is able to produce both types of aflatoxin [9,23]. Determination of the aflatoxin production capacity from the emission of fluorescence on the CAM medium and the quantification of aflatoxins in rice by HPLC. The local isolate F_3 of *Aspergillus* section *Flavi* shares characteristics with the reference strain UBOCC-A-106031 *A. flavus*. We have classified it in this species. The results relating to the morphological criteria show that this isolate corresponds to those of *A. flavus*. According to several mycologists *A. flavus* is the most frequently encountered contaminant in cereals, especially in humid and hot countries [2,6]. Furthermore, the analysis of aflatoxins in the R441 rice sample in which we isolated F_3 revealed the presence of AFB only. Our results showed similar observations to those of Katsurayama et al. [29] who demonstrated that *A. flavus* is not able to synthesize AFG. Thus, the high dose of AFB in the rice samples could be explained by the fact that this latter is synthesized by *A. flavus* and *A. parasiticus* while AFG is only produced by *A. parasiticus*.

Aflatoxin production ability performed by fluorescence under UV light of local isolates and reference strains by cultivating them on CAM were in concordance with those obtained by HPLC quantification. All isolates showing blue fluorescence emission. These findings are similar to those observed by Alkhersan et al. [30] who confirmed emission of blue fluorescence around colonies of *A. flavus* aflatoxinogenic isolated from poultry feed by ammonia vapor production on CAM medium.

Quantitative aflatoxin analysis by HPLC revealed that three of the four samples analyzed were contaminated with aflatoxins. The highest dose of AFs was 2.40 ± 0.04 µg/Kg and was detected in sample R441, AFB1 was present in two samples and its highest value was 2.18 ± 0.04 µg/Kg. Which was above the EU regulation 1881/2006 for AFB1. This legislation limits for peanuts, cereals and derived from cereals other than maize and rice was 2 µg/kg for AFB1, 4 µg/kg for AFs. Nevertheless, AFs content in our samples was lower than this limit. But if during the manufacturing process of infant flours this aflatoxin is not denatured, it could be found in the flours and cause disease in newborns, The EU Regulation 1881/2006 limits for infant formulas are 0.1 µg/kg for AFB1. Indeed, an ingestion of only 20 µg/Kg may cause irreversible damage to the liver, especially in infants. The Acceptable Daily Intake (ADI) or Tolerated Daily Intake (TDI) which designates the theoretical maximum quantity of substance which can be administered, daily and throughout life, to an individual

(sensitive or not) without risk of causing occurrence of adverse health effects is 0.15 ng/Kg/Day for AFB1 [31]. It is for this reason that European standards are evolving towards zero tolerance in foodstuffs for aflatoxins. The contamination of our samples with aflatoxins could be explained by the presence of toxigenic strains *Aspergillus* section *Flavi*. Indeed, these latters are the fungal contaminants most frequently encountered in cereals and which secrete aflatoxins there when environmental conditions (temperature, humidity, pH, etc.) are met, as is the case with the pH of our relatively wet rice samples which are slightly acidic. Several bad practices in Burkina Faso can explain the level of cereals contamination. Among others, there are bad cultural practices, storage, threshing of poorly dried rice leading to excessively high breaking rates facilitating the proliferation of *Aspergillus* section *Flavi* [5]. Our farmers often find it difficult to export their crops to countries where aflatoxin regulations are very strict and very large stocks are often rejected [3].

5. CONCLUSION

In this study, we isolated twenty strains of mold in four samples rice intended for infant flour production, among which three strains of *Aspergillus* section *Flavi*. The morphological identification as well as the qualitative determination of aflatoxin production ability was confirmed by the results of the quantitative analysis of aflatoxins by HPLC which also revealed three samples were contaminated by aflatoxin. However, this identification should be confirmed by modern methods such as PCR and household genes sequencing. Several studies in the world on the incidence of mycotoxins in rice report a large number of samples contaminated with these metabolites of *Aspergillus* section *Flavi* at high levels, especially in countries with lower economic resources and where rice constitutes the main nutritional source of the diet. Given the danger and the almost non-existent data available on the presence of mycotoxins in foodstuffs in general and cereals in particular in Burkina Faso, the present study aims to raise awareness of the presence of these fungal metabolites in rice and probably in infant flour. Good production, processing practices as well as Hazard Analysis and Critical Control Points (HACCP) from production to consumption must be applied in order to protect the health of consumers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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