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# Levels of fumonisins B1 and B2 in two (2) stored commodities: the case of sorghum (*Sorghum bicolor*) and millet (*Pennisetum spp*) intended for human consumption

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## Abstract

The aims of this study is to evaluate the level of fumonisin B1 and B2 in sorghum (Sorghum bicolor) and millet (Pennisetum spp). To do this, a method for assaying FB1 + FB2 has been validated and the quantification limits of FB1 and FB2 obtained are respectively 17.5 µg / kg and 22.1 µg / kg. The extracts were purified on immunoaffinity cartridges. The detection and quantification of fumonisins was carried out by HPLC equipped with a fluorescence column detector RP 18. One hundred (100) samples of sorghum and millet were collected in three (3) localities in Côte d'Ivoire namely Korhogo, Boundiali and Abidjan. 45% of the samples taken were fumonisin contaminated, of which 32% had FB1 levels with an average of 40.71 ± 5.28 µg / kg, compared to 13% for a presence in FB1 + FB2 at an average concentration of 206.71 ± 17.96 µg / kg . These detected levels were all below the maximum tolerable level set at 4000 µg FB1 + FB2 / kg in raw cereals according to EC Regulation 1126/2007.

## 1. Introduction

Sorghum and millet are crucial commodities of the world food economy as they contribute to household food security in many of the poorest and most food-insecure regions [1]. For many developing countries, these cereals represent the essence of the diet of low-income rural populations [2]. Generally, sorghum and millet are consumed as whole grain or transformed into flour, from which traditional semolina is prepared. In Côte d'Ivoire, the annual production of sorghum and millet is estimated at approximately 100 000 tons according to the Ministry of Agriculture DSDI / MINAGRI [3], and are used for the preparation of porridge for children and adults as well as the production of a local beer, tchapalo, coming from the northern regions of the country. However, these cereals may be damaged during production, harvesting, transportation, drying, storage, processing and distribution. These deteriorations are of various natures. They may coming from the activity of mycotoxin-producing fungi such as Trichothecenes, fumonisins, Aflatoxins etc., and their propagation depends on two climatic factors which are temperature and humidity [1]. Among these mycotoxins, Fumonisins pay more attention. These are secondary metabolites produced in cereals by pathogenic fungi, mainly *F. verticillioides* and *F. proliferatum* [4]. Currently, more than 15 fumonisin homologues have been identified [5]. Among them, fumonisin B1 (FB1) is the most toxic form and, in many cases, co-occurs with other congeners such as fumonisin B2 (FB2) and B3 (FB3) [4]. The MLs are intended to protect health so that fumonisin exposure is less than the provisional maximum tolerable daily intake (TDI) of fumonisin of 2 µg / kg body weight / day set by the Joint FAO / WHO Committee. Expert Panel on Food Additives (JECFA) [6]. Indeed, these compounds are considered probable carcinogens in humans by the International Agency for Research on Cancer IARC [7], because fumonisin is associated with neural tube defects in infants and oesophageal cancer [8]. In view of the carcinogenic nature of

these mycotoxins, a maximum tolerable level of fumonisins (B1 + B2) has been fixed for cereals and cereal products, by Regulation EC / 1126 (2007)[9], in order to prevent the occurrence of risk of consumer exposure to these fumonisins. In Côte d'Ivoire, in spite of the significant contribution of sorghum and millet to food and their significant contribution to the economy, these cereals have not been sufficiently explored by research, and the available scientific does not shed light on the level of contamination of these products by mycotoxins in general and fumonisins in particular. Thus, the objective of this study is to determine the level of mycotoxin contamination including fumonisins B1 and B2 in millet and sorghum stocks in order to contribute to the assessment of the health risk of Ivorian populations to mycotoxins.

## 2. Materiel and Methods

*2.1. Study areas*

For these works, three study areas were selected for sampling. These are the localities of Korhogo, Boundiali and the district of Abidjan (Figure 1). Korhogo and Boundiali localities are among the largest areas of millet and sorghum production in Côte d'Ivoire DSDI / MINAGRI [3].


Legend

Source: MINAGRI, [3]

: Savannah region (large production areas)

: Regions of cultivation of millet and sorghum

: Study and sampling sites

**Figure 1:** Map of Côte d'Ivoire showing production and sampling sites for sorghum and millet (modified figure)

These two localities are limited: to the north by the 10th parallel and to the south by the 9th parallel, to the east and west between 6 ° and 7 ° west longitude for the map of Boundiali and between 5 ° and 6 ° west longitude for the map of Korhogo[10].
In northwestern Côte d'Ivoire, the climate is tropical sub humid. According to the observations of Baudeau and Sayol[8], it is characterized by the following succession:
-A dry season that runs from November to May. January is usually the driest month. The months of December, January, February and even March are subject to harmattan.
- A rainy season that lasts from June to October. The rainiest months are July, August and September. Maximum rainfall is observed in August. The annual average temperature is 26.7 ° C in Korhogo.
The third zone of study, the district of Abidjan is a zone of high consumption of millet and sorghum, considering its density of population of 1475 inhabitants / km2[11].
It is located in the south of Côte d'Ivoire and located between latitudes 5 ° 00 and 5 ° 30 North and longitudes 3 ° 50 and 4 ° 10 West. The city of Abidjan covers an area of 57,735 hectares [12].

The south of the country has a subequatorial climate characterized by temperatures of low amplitudes of (25 ° C to 30 ° C), a high humidity (from 80 to 90%) and abundant precipitations, which reach in Abidjan 1766 $mm^3$. This area has two dry seasons and two wet seasons. The long dry, hot season is interrupted by a few rains and extends from December to April. The short dry season covers the months of August and September. As for the rainy seasons, they spread out May at July for the large one and from October at November for the small one[10].

*2.2. Sampling*

The sampling technique used for mycotoxin analysis is based on EC Directive 2007/1126[9] and the standards (ISO 3696, 1987, ISO 5725.1866)[13-14]. For this study, the harvesting of stored commodities involved millet and sorghum in ground grain (husked) marketed in the 3 localities of the country, Korhogo, Boundiali, and Abidjan. An average of 30 to 40 test shots, of 1 kg each, were randomly taken from the sorghum and millet batches at each site. Each batch consists of a set of 12 bags of 50 kg on average stored in a warehouse or shed. Each test portion was finely milled in a mill and a 25 gram sub-sample was taken for the analysis of FB1 + FB2 by high performance liquid chromatography (HPLC). A total of 100 samples were taken based on the importance of production and consumption requirements relative to the sampling sites (Table 1). These different samples were packed in stomachers bags and stored in a cold room at -20 ° C in the laboratory until analysis.

**Table 1:** Number and types of samples taken at each localities

| | Localities | | | |
|---|---|---|---|---|
| **Designation** | **Korhogo** | **Boundiali** | **Abidjan** | **Total** |
| Red Sorghum grain | 10 | 5 | 5 | 20 |
| White grain Sorghum | 5 | 5 | 5 | 15 |
| Ground red Sorghum | 5 | 5 | 5 | 15 |
| Ground white Sorghum | 5 | 5 | 5 | 15 |
| Millet | 10 | 5 | 5 | 20 |
| Ground millet | 5 | 5 | 5 | 15 |
| Total samplers | 40 | 30 | 30 | 100 |

*2.3. Determination of Fumonisins B1 and B2*

According to the method [15], a sample of 1 kg of grain cereal was finely milled. An amount of 25g of this ground material was taken and added to 100 ml of an acetonitrile / methanol / water mixture (25/25/50, v / v / v). The mixture was homogenized with an ultra-turax for 4 minutes and centrifuged at 5000 rpm for 5 min at + 4ºC and then filtered on paper (whatman) in 100 mL Erlenmeyer flasks. To 10 ml of this filtrate a volume of 40 ml of PBS

buffer (buffered phosphate salt solution) was added. The fumonisin-specific immunoaffinity columns are removed from the refrigerator for at least 30 minutes at room temperature prior to use. These columns were then emptied of their storage liquid and were packaged with 5 mL of PBS buffer using a vacuum pump at a rate of 1 mL / min so as not to leave the columns dried. Subsequently, 10 ml of the filtrate were removed and passed to the purification on the immunoaffinity column at a flow rate of 1 ml / min. The column was washed with 10 mL of the same buffer. FB1 or FB2 was eluted with 1.5 mL of methanol followed by 1.5 mL of double distilled water. A volume of 2.8 mL is collected in a 4 mL recovery tube. The eluate is recovered and evaporated in the dark by Rotavapor-215 rotary evaporator. The dry extract is taken up in 200 µL of an acetonitrile / water (50/50, v / v) vortex mixture for 30 seconds. A volume of 100 µL of purified extract was removed and added to 100µLof derivatization solution OPA-2Mercaptoethanol in order to make the fumonisin molecule fluorescent. The eluate is injected into the high performance liquid chromatography, 2 minutes after the addition of the derivatizing agent so as to reduce any variability. The chromatographic chain is equipped with a fluorescence detector (Shimadzu RF-10AXL) at 335 nm excitation wavelength and 440 nm emission wavelength, fixed for fumonisin analyzes.
The pump used, is of type (LC-20AT) with a column C-18 ODS-3-5, 200 L x 4 mm (Intersil). The flow rate is 1.00 mL/minute in Socratic mode. The injection volume is 100 µL and the eluate analysis time is 25 minutes. The mobile phase consists of a mixture of methanol/buffer $NaH_2PO_4$ 0.1 M proportion (75/25, v / v) adjusted to pH 3.35 with hydrochloric acid. The quantities of FB1 and FB2 are determined by linear regression by comparing the surface obtained with that obtained with ranges of standards of known concentrations.

*2.4. Determination of concentrations*

Determine the concentrations of FB1 or FB2 (in ng), contained in the test solution injected into the HPLC column, from the calibration curve.
The concentration (CFB1 and CFB2) is calculated as fumonisins in micrograms per gram (µg / g) from the following formula:

$$CFB = \frac{M_A x 10^{-3}}{M_B x 10^{-3}} = \frac{M_A}{M_B} = \frac{M_A}{125}$$

With:
MA: mass of FB1 (in ng) continuous in the test of the matrix injected into the column and determined from the calibration line;
MB: mass of the matrix (in mg) injected into the column (125 mg);
1/1000: AD conversion factor and MB masses from ng to µg and from mg to g, respectively.

*2.5. Statistical analyzes*

Statistical analyzes were performed using the XLSTAT version 7.5 statistical software. For the comparison of the k samples, a multiple comparison test for the variable Tukey (HSD) / Analysis of differences between groups with a 5 % confidence interval was performed.

## 3. Results and discussion

Table 2 presents the results of analyzes of millet and sorghum samples taken from all three Localities.

**Table 2:** Proportion and levels detected in fumonisins in contaminated samples

| Requested elements | FB1 | FB1+FB2 | < LQ | ND |
|---|---|---|---|---|
| Contaminated samples in (%) | 32 | 13 | 52 | 3 |
| Content detected in (µg / kg) | [17.5-156.22] | [181.02-297.27] | [5.83-17.5[ | 0 |

LQ = 17, 5µg/kg; ND: No detected

Of the 100 samples collected and analyzed, 32% are contaminated with FB1, the contents of which are between 17.5 and 156.22 µg / kg against 13% contaminated with FB1 + FB2 at levels ranging from 181, 02 to 297.24 µg / kg, then 52% have contents below the limit of quantification (LQ = 17.5 µg / kg) and 3% of the samples are undetected. No samples have FB2 contents only.

A total of 45% of all millet and sorghum samples are contaminated with type B fumonisins and 52% have traces of FB with levels above the Limit of Detection (LD) and below the Limit Quantification (LQ). In fact, these levels below the LQ cannot be considered reliable because the uncertainty associated with the measurement is greater according to Quebec's center of expertise in environmental analysis [16].
The results obtained in this study suggest that traces of fumonisins can be found in sorghum and millet. This results is conformity with several work of authors' such as [17; 18; 19] that claim a naturally occurring sporadic occurrence of fumonisins in sorghum, although these fumonisins have been detected mainly in maize and maize products. Within sight of the detected levels, it appears that the samples analyzed have levels that comply with the regulations. Indeed, the European Commission has set a limit of 4000 µg fumonisin / kg in raw cereals for all fumonisin B1 + fumonisin B2 EC Directive 2007/1126[9].
For the levels detected in each matrix, the following table presents the proportions and levels of contamination of millet and sorghum in all three localities.

**Table 3:** FB1 + FB2 contamination levels detected in the millet and sorghum samples in the three localities

| | Matrices | **MIL** | | | | **SORGHO** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Types | FB1 | FB1+FB2 | < LQ | ND | FB1 | FB1+FB2 | < LQ | ND |
| | (%) Contaminated samples | 20 | 20 | 60 | 0 | 39 | 9 | 49 | 3 |
| Content detected in µg / kg | **Minimum** | 17.5 | 181.03 | | | 17.52 | 66.19 | | |
| | **Average** | **25.09 ± 3.88** | **241.11 ± 41.23** | | | **35.24 ± 41.71** | **166,36 ± 83.11** | | |
| | **Maximum** | 28.67 | 297.15 | | | 156.22 | 268.44 | | |

LQ = 17, 5µg/kg; ND: No detected

The presence of fumonisins is observed at the level of the two cereals which present a certain percentage of samples contaminated as well with FB1 as with FB1 + FB2. The results obtained indicate that 40% of millet samples are contaminated with fumonisin B compared with 48% for sorghum. Tukey (HSD) tests indicate that there is no significant difference between FB1 + FB2 contaminations of millet and sorghum (p> 5%), as well as levels of contamination in FB1 (p> 5%) (See Tables 4 and 5).

**Table 4:** Result of the multiple comparison test for the Mil-Sorgho variable (FB1 + FB2)

| Modalities | Difference | Reduced difference | Critical value | Pr. > Diff | Significant |
|---|---|---|---|---|---|
| MIL ~ SORGHO | 38.031 | 1.267 | 2.262 | 0.237 | No |

Critical value of Tukey's d: 3,199

**Table 5:** Result of the multiple comparison test for the Sorghum-Mil variable (FB1)

| Modalities | Difference | Reduced difference | Critical value | Pr. > Diff | Significant |
|---|---|---|---|---|---|
| SORGHO ~ MIL | 29.448 | 1.705 | 2.048 | 0.099 | No |

Critical value of Tukey d: 2,897

These tests indicate that the groups namely the extent of millet and sorghum contamination by FB1 and FB1 + FB2 are not significantly different. At each sampling site (locality), the following Tables present the proportions and contamination levels in FB1 + FB2 detected in the samples per cereal matrix.
The proportion of millet samples contaminated with FB1 + FB2 is 70% in Abidjan with an average content of 241.11 µg / kg. However, no FB2 contamination was found in samples from Korhogo and Boundiali. We also observe a contamination of millet by FB1 at a proportion of 13.33% in Korhogo and 40% in Boundiali samples. The average contents are 26.85 and 25.04 µg / kg respectively for Korhogo and Boundiali.

**Table 6:** proportions and levels of FB1 + FB2 contamination of millet in each locality

| | Localities | ABIDJAN | | | KORHOGO | | | BOUNDIALI | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Types (%) | FB1 | FB1+FB2 | < LQ | FB1 | FB2 | < LQ | FB1 | FB2 | < LQ |
| | Contaminated samples | 10 | 70 | 20 | 13.33 | 0 | 86,67 | 40 | 0 | 60 |
| Content detected in µg / kg | Minimum | 17.5 | 181.03 | | 25.09 | | | 21.9 | | |
| | Average | 17.5 ± 34.75 | 241.11 ± 34.75 | | 26.85 ± 1.76 | | | 25.04 ± 1.59 | | |
| | Maximum | 17.5 | 297.25 | | 28.62 | | | 28.63 | | |

LQ = 17,5µg/kg

The Tukey test (HSD) indicates a significant difference between Abidjan and the two localities (Korhogo and Boundiali) (p <5%) but there is no difference between Boundiali and Korhogo (p> 5%). The result of the multiple local comparison test for millet is shown in Table 7.

**Table 7:** Result of the multiple comparison test by location for millet

| Modalities | Difference | Reduced difference | Critical value | Pr. > Diff | Significant |
|---|---|---|---|---|---|
| Abidjan ~ Korhogo | 156.154 | 6.278 | 2.461 | < 0,0001 | Yes |
| Abidjan ~ Boundiali | 149.748 | 5.350 | 2.461 | < 0,0001 | Yes |
| Boundiali ~ Korhogo | 6.406 | 0.249 | 2.461 | 0,966 | No |

Critical value of Tukey d: 3.481

Indeed the table of the classification and groupings of the not-significantly different localities shows that the extent of millet contamination in FB1 + FB2 is higher than in Korhogo and Boundiali.

**Table 8:** Classification and grouping of non-significantly different groups for millet

| Modalities | Average | Combinations | |
|---|---|---|---|
| Abidjan | 180.555 | A | |
| Boundiali | 30.806 | | B |
| Korhogo | 24.401 | | B |

Cities with the same letter are not significantly different

In fact, the Table of Classification and groupings of non-significantly different groups shows that contamination levels of millet in FB1 + FB2 are higher in Abidjan (group A) than in Korhogo and Boundiali (group B).

**Table 9:** proportions and levels of FB1 + FB2 contamination of sorghum in each locality

| | Localities | ABIDJAN | | | KORHOGO | | | BOUNDIALI | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Types (%) | FB1 | FB1+FB2 | < LQ | FB1 | FB1+FB2 | < LQ | FB1 | FB1+FB2 | < LQ |
| | Contaminated samples | 33 | 6 | 61 | 58 | 8 | 34 | 28 | 16 | 56 |
| Content detected in µg / kg | Minimum | 17.52 | 140.91 | | 18.5 | 66.19 | | 37.54 | 166.36 | |
| | Average | **18.5 ± 0.98** | **140.91 ± 0.00** | | **38.4 ± 17.12** | **66.19 ± 0.00** | | **75.15 ± 24.03** | **251.75 ± 16.68** | |
| | Maximum | 21.84 | 140.91 | | 156.22 | 66.19 | | 99.19 | 268.44 | |

LQ = 17,5µg/kg

Regarding sorghum, we record contaminated samples in FB1 + FB2 at the 3 localities, 6% in Abidjan with an average grade estimated at 140.91µg / kg, 8% in Korhogo with an average grade of 66.19 µg / kg and 16% at Boundiali with an average content of 251.75 µg / kg, one of the highest levels detected in the samples.

The Tukey multiple comparison test (HSD) reveals a significant difference between Boundiali and Abidjan (p <5%). The other localities namely Boundiali and Korhogo, Abidjan and Korhogo are not significantly different for sorghum (p> 5%). The result of the multiple comparison test per locality for sorghum is shown in Table 10.

**Table 10:** Result of the Multiple Comparison Test by Location for Sorghum

| Modalities | Difference | Reduced difference | Critical value | Pr. >Diff | Significant |
|---|---|---|---|---|---|
| Boundiali ~ Abidjan | 46.196 | 2.876 | 2.402 | 0.015 | Yes |
| Boundiali ~ Korhogo | 26.319 | 1.724 | 2.402 | 0.204 | No |
| Korhogo ~ Abidjan | 19.877 | 1.321 | 2.402 | 0.389 | No |

Critical value of Tukey d: 3.397

The classification and grouping of locations not significantly different for sorghum in Table 11 indicates that sorghum samples from Boundiali are more contaminated with FB1 + FB2 compared to other sampling sites. There are three levels of contamination that are category A, category AB and category B. Category A corresponding to the highest levels, it groups the samples of the city of Boundiali. Category AB, corresponding to an intermediate situation, it groups the samples of the city of Korhogo. Finally, category B corresponds to the lowest levels, it takes into account the samples from the city of Abidjan (Table 11).

**Table 11**: Ranking and grouping of locations not significantly different for sorghum

| Modalities | Average | Combinations | |
|---|---|---|---|
| Boundiali | 72.535 | A | |
| Korhogo | 46.216 | A | B |
| Abidjan | 26.339 | | B |

Cities with the same letter are not significantly different

This state of contamination with this type of mycotoxin observed during this study could be due to the type of storage commonly used or the long storage time characterized by inadequate storage conditions observed in the localities from which our samples of millet and sorghum come from. Indeed, these foods are kept without adequate packaging at the commercial surfaces, in an environment exposed to the humidity of the air and excessive heat. In fact, [20] point out that the storage of grains harvested under high humidity conditions leads to a further accumulation of fumonisins. The types of storage used at each north localities namely the attics and the hangars, could favor the occurrence of the mycotoxins in the foodstuffs exposed to the attacks of insects and other pests. The relatively low FB1 levels detected in some samples could be for cereals that come directly from the recent harvest. On the other hand, level of fumonisins above average may be due to inadequate storage and storage conditions which are influenced by physical factors such as humidity and temperature. Indeed, these poor storage conditions and physical factors (high temperature and high humidity) cause a very significant losses in storage of the sorghum and millet observed by Pushpamma et *al*., [21].

The statistical and descriptive tests in this study reveal that the presence of FB1 and FB2 is not related to the types of matrices (sorghum or millet). In other words, the contamination does not depend on the nature of the millet or sorghum samples for these cases studied.

However, studies by [22] show that the extent of fumonisin contamination of raw cereals varies with geographical location, farming practices, and the genotype of the cereal that determines the susceptibility of seedlings. Cereal with fungal invasion and insects during the growing phase of corn, sorghum and millet in the fields. In fact, the fumonisins found in cereals seem to be produced in the fields, on corn, millet and sorghum by a fungus *F. verticillioides*. Moreover, the presence of Fusarium species producing fumonisins is worsened by attacks of pyrale (*Ostrinia nubilalis*) an insect which causes lesions in the ears and stems, constitute entrance doors for these mushrooms described by Gatch and Munkvold in 2002; Alma et al., In 2005 cited in the AFSSA report (2009)[23]. Therefore, some agronomic practices are also involved in the development of *F. verticillioides* and fumonisin production and the fumonisin content may be related to the duration between the harvest and the drying of the cereal grains.

## Conclusion

The results obtained in this study suggest that fumonisin traces may be found in sorghum and millet that are vulnerable to fumonisin contamination. Forty-five (45) % of the samples analyzed yielded fumonisin B1 + B2 levels with contamination levels in accordance with European recommendations for maximum tolerable levels of fumonisin B in foods for human consumption. Our study sites show no significant difference in the extent of contamination of these cereals with fumonisins in Côte d'Ivoire. Given their acute toxicity, especially the most toxic FB1 analogous fumonisins, it would be important to assess the level of contamination of foods and products derived from these two cereals, since the decontamination of products contaminated by these toxins are very difficult. These results could serve as a basis and scientific data for a food risk assessment related to the consumption of these foods by the African populations.

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