Aspergillus flavus and Aflatoxin B1 in maize flour sold in the markets of Bobo Dioulasso (Burkina Faso)

Cherileila THIOMBIANO[1,2,*], Abdallah DAO[3], Isidore Tiandiogo TRAORE[1,2], Souleymane DIARRA[3], Salifou Cheik Abbas ZERBO[1], Sougrinoma Achille OUEDRAOGO[1], Alain HIEN[1,2], Tarwendpanga Bernadette PICBOUGOUM[1] et Hervé HIEN[1]

Abstract

Maize, widely consumed in Burkina Faso remains a substrate appreciated by aflatoxigenic moulds. The objective of this study was to give the frequency of aflatoxins B_1 and *Aspergillus flavus* in maize flour sold in Bobo Dioulasso. We conducted a cross-sectional study in Bobo-Dioulasso. From each of the 53 maize flour sellers, 150 g of maize flour was collected. A sanitary analysis was conducted to determine the rate of *Aspergillus flavus* contamination. A rapid detection kit was used for Aflatoxin B_1 screening. The amounts of aflatoxin B_1 detected were grouped into 4 classes: < 5 ng/g, [5- 10 [ng/g, [10- 20[ng/g and ≥ 20 ng/g, and compare to the codex Alimentarius recommendations. The sanitary analysis showed that 90.57% of samples were contaminated by *Aspergillus flavus*, with an average contamination rate of 60.18%. This rate was significantly different between central and peripheral markets (p-value=0.02). Aflatoxin B_1 was detected in 94.33% of samples, and 58.49% were above the codex Alimentarius recommendations for Aflatoxins B_1(10 ng/g). The amounts of aflatoxin B_1 increased with the average contamination rate by *Aspergillus flavus* ($p < 0.001$). About 60 % of maize flour sold in the markets of Bobo Dioulasso (Burkina Faso) is unsuitable for consumption.

Keywords: Maize, flour, Aflatoxin B_1, *Aspergillus flavus*, Bobo-Dioulasso

Introduction

In Burkina Faso, farming is practiced by about 86% of the population (1). One of the most important crop productions in this country is maize (2). It is the most consumed cereal in the human diet, but also the most used in poultry, pigs, and cattle breeding. Unfortunately, sellers face problems with post-harvest storage of maize. One of the most common

[1] Laboratoire de Recherche, Centre Muraz, Institut National de Santé Publique (INSP). Bobo Dioulasso, Burkina Faso

[2] Institut Supérieur des Sciences de la Santé (INSSA), Université Nazi Boni, Bobo-Dioulasso, Burkina Faso

[3] Institute de l'Environnement et de Recherche Agricole (INERA), Centre National de la Recherche Scientifique et de la technologie (CNRST). Bobo Dioulasso, Burkina Faso

*Corresponding author: Cherileila THIOMBIANO, Tel: 00226 71340505 email : leiryza@yahoo.fr,

and the least discussed problem remains the aflatoxins contaminations, resulting from crops attacks by various species of microscopic fungi such as molds.

A large number of mould species belonging mainly to the three very common genera, *Aspergillus, Penicillium* and *Fusarium* present in ambient air, soil, and agricultural crops are able to grow on cereals to synthesize and excrete mycotoxins (3). The most commonly found is *Aspergillus flavus* species. It is mainly found in tropical and subtropical regions, with high temperature and humidity, combined with inappropriate storage practices, contribute to its development (4).

In the USA the accepted level for human consumption is 20 ng/g (5) and for the European Union, the maximum acceptable total aflatoxins (B_1, B_2, G_1 and G_2) in nuts, dried fruits, cereals and spices is 4 ng/g (6). According to the Codex Alimentarius, the maximum limit for aflatoxins in almonds is 10 ng/g (7).

Aflatoxins are a class of carcinogenic mycotoxins produced by Aspergillus fungi, which are widely distributed in nature. Aflatoxin B1 (AFB1) is the most toxic of these compounds and its metabolites have a variety of biological activities, including acute toxicity, teratogenicity, mutagenicity and carcinogenicity, which has been well-characterized to lead to the development of hepatocellular carcinoma (HCC) in humans and animals (8)African continent loses approximately US$670 million each year in export revenue due to aflatoxins contamination. Aflatoxins are harmful to human and animal health. They cause about 30% of all liver cancers cases worldwide, with the highest incidence (over 40%) in Africa. They are also associated with immuno-suppression and growth deficiency in children (9).

Maize produced in developing countries contains some level of aflatoxins (10). Indeed, Studies following an acute outbreak in four districts of Kenya showed levels of 1 to 46400 µg/kg total aflatoxins (11). Furthermore, Gnonlonfin et al. (12) found aflatoxins levels in maize in Benin, Ghana and Zambia of 2 to 2500 µg/kg, 20 to 355 µg/kg and 1 to 109 µg/kg respectively.. In a recent study only 4.6% of maize samples from four West African countries had levels above 20 ng/g (13).

In Burkina Faso, the presence of aflatoxins were reported in maize (14, 15), while few studies have addressed this issue in the case of maize in Bobo Dioulasso. The aim of this study was to determine the frequency of maize flour contaminated with aflatoxin B_1 and *Aspergillus flavus* sold in markets of Bobo-Dioulasso.

I. Materials and methods

1.1. Study design and setting

This observational cross-sectional study was carried out in Centrre Muraz located in Bobo-Dioulasso, the economic capital of Burkina Faso.

1.2. Sampling and data collection procedures

From June to July 2021 a completed list of all the markets in Bobo-Dioulasso was established.

Since the conditions of maize flour storage may vary according to the location of the markets in the city (central or peripheral district), the markets were classified into two groups: «central» and «peripheral». Then, a random sample stratified on the geographical location of 11 markets (6 central and 5 peripheral) was selected.

We used the OpenEpi software to calculate the sample size (16). For that purpose, the proportion of maize flour samples with an aflatoxin concentration above the 10-ng/g threshold was estimated at 30%, with a precision of 13%, with no cluster effect and a 10% non-response rate. This results in 53 samples as the minimal sample size. A proportional allocation was made.

From each seller 150 g of maize flour was taken in sterile polyethylene bags (100 x150 mm), which were immediately stored at -20°C for analysis.

For ethical reasons, free, informed and individual consent was obtained from each participant. The anonymity and confidentiality of the information collected were respected.

1.3. Laboratory procedures

Aspergillus flavus isolation in maize flour

Aspergillus flavus isolation was performed with Potatoes Dextrose agar (PDA) (17). Approximately 43 g of PDA was mixed with 1 liter of distilled water and homogenized until complete dissolution on a hot plate. The mixture was then sterilized with autoclave at 121°C for 25 minutes. After cooling at about 45°C, 1 ml of streptomycin was added to prevent the growth of non-target pathogens. The agar was then poured into Petri dishes under aseptic conditions, under the hood at 25 ml per dish. One or two pinches of flour of the same sample was deposited on five spots of the agar, with six replicates for each sample. Then, the Petri dish was sealed and incubated at 25°C. After 48h to 72h, Petri dishes were observed with magnifying glass and microscope. Infection rates of maize flour were computed following the formula: $(\sum ni * 100)/30$ with *ni*, the occurrence number of the mushroom in each Petri dish.

Qualitative detection of Aflatoxin B_1 in Maize flour

Aflatoxin B_1 detection was performed using the rapid test kit N.K. Biotech (catalogue number: **AFL**B_1**-D205F1**), with a sensitivity of 5 ng/g (5 µg/Kg), 10 ng/g (10 µg/Kg), 20 ng/g (20 µg/Kg) (18). One gram of the testing flour was mixed with 4 ml of kit solution A in a 5 ml tube. The mixture was then shaken vigorously for 3 minutes and centrifuged at 1789 x g for 5 min. After centrifugation, 100 µl of the supernatant was collected and placed in a 1.5 ml tube. 100 µl of $AFLB_1$ PBST buffer from the kit was added and shaken until complete homogenization. 100 µl of this solution is used for the test according to the manufacturer's instructions (18).

1.4. Study variables

Outcomes variables were the rate of *Aspergillus flavus'* contamination and the rate of Aflatoxin B_1 contamination. Maize flour contamination rate with *Aspergillus flavus* was defined as the number of times the mould grow on the number of depots made. Using different cut-off of the test, the rate of Aflatoxin B_1 contamination were grouped into 4 classes: < 5pp, [5, 10 [ng/g, [10, 20 [ng/g, and ≥ 20 ng/g.

1.5. Data analysis

Standard statistics were used to summarize the qualitative variables, their frequencies and 95% confidence interval (95%CI). Analysis of variance (ANOVA) was used variance to compare contamination rates between the markets and between the central and peripheral areas.

A simple linear regression with a categorical exogenous variable was used to assess the amounts of aflatoxin B_1 increase according to the average contamination rate by *Aspergillus flavus*. All analyses were performed using R4.0.2 software.

II. Results and Discussion

In the 11 markets selected in the city, 53 samples collected from 53 sellers were included in the study. Among them, 28 (52,83%) were from central markets.

2.1. Maize flour contamination rate with *Aspergillus flavus*

The proportion of maize flour samples contaminated by *Aspergillus flavus* was 90,57% (CV = 79.58-96.47), with an average contamination rates of 60,18% (CV = 51.22-69.15). This average contamination rate varied significantly between the markets (from 18, 33% to 92,22%) p =0.009. (**Fig 1**). Regarding the contamination of maize flour by *Aspergillus flavus*, our result in Bobo-Dioulasso was not in line with the finding of Sebok and al. (2016), who reported that *Aspergillus flavus*, was isolated from 42.3% of maize sample in 2013, and from 34.7% maize samples in 2014 in Hungarian (19). The preharvest, and postharvest storage conditions was probably better in hungarian than in Bobo-Dioulasso and could justified the higher frequency in Bobo-Dioulasso. It is important to find some solutions to inhibit growth of *Aspergillus flavus*. Indeed some studies revealed that different saprophytic yeasts strains are able to inhibit growth and aflatoxin production of *Aspergillus flavus* both in vitro and in vivo. (20, 21).

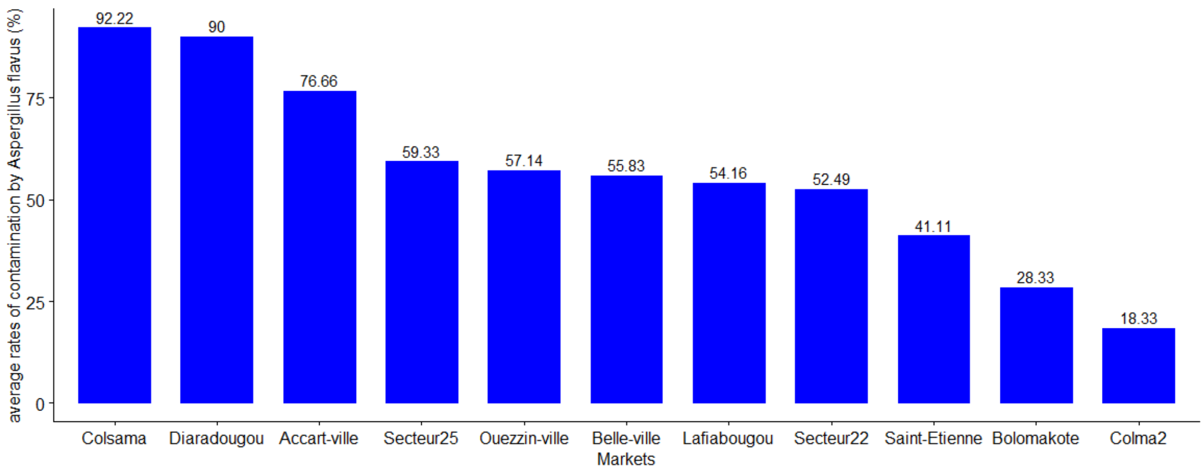

Figure 1. Maize flour contamination rates by *Aspergillus flavus* depending on the market to the markets in Bobo-Dioulasso (Burkina Faso).

2.2. Contamination rate depending to the areas (central or peripheral)

The contamination average rate was 69,76% for market from central area and 49,46% for market from peripheries area. . Flour samples from central markets were the most contaminated by *Aspergillus flavus* and the difference between markets from central area and those from peripheral area was statistically significant (p = 0.02) showing that people in central area was more exposed than those in peripheral area.

2.3. Maize flour contamination by Aflatoxin B1

94,34% of samples was contaminated by aflatoxins where, more than half (58.49%) of maize flour samples were above the codex Alimentarius recommendations for Aflatoxins B_1(10 ng/g), and almost 38% were above 20 ng/g. (**Fig 2).** These results were consistent with those found by Ware et al. (2017) who reported in their study a frequency of 83.9% for aflatoxin B1, in commercial infant formulas produced in Burkina Faso (22). Another study conducted in Burkina Faso and Mozambique by Benedikt Warth et al. showed that Aflatoxin B(1) was observed more frequently in maize in Burkina Faso with the half (50%) of the maize samples contaminated with aflatoxin B_1 (50% incidence) (23). Kpoda et al (2022) found that in Burkina Faso, 42.03% of maize sample were contaminated with aflatoxins (14). Our samples were collected from June to July where maize flour are exposed to hot climate. This could explained the higher result we found in this study. However, all these studies conduct in Burkina Faso, showed that maize sample was contaminated by aflatoxins. The presence of mycotoxins is related to climatic conditions, inappropriate storage practices and could justify the results of these studies. Exposure to toxigenic fungi and their metabolites has been linked to child growth and weight impairment (9).

The analyses highlighted flour samples with levels of AFB_1 that exceeded the standards tolerated by the Codex Alimentarius. To assess the safety of food products, we refers to the standards of the Codex Alimentarius as Burkina Faso is a member of the codex commission(7). Among the samples contaminated with aflatoxins, almost 60% of samples had Aflatoxins concentration above Maximum Limit (10 ppb) according to Codex Alimentarius. Consuming these flours expose to risk of developing liver cancer (23) or risk of stunting for children. With regard to the dangerous aspect of this toxin, these flours would be unsafe for consumption and therefore should be withdrawn from the marketing circuit. However, given the importance of maize in the food habits of burkinabé, and given the conditions of production and distribution of this cereal, currently it will be hard to prohibit the consumption of these meals (24). To be effective, measures to reduce or eliminate aflatoxins in maize will have to be part of an overall framework to improve storage conditions for agricultural products in Burkina Faso.

Because of the high prevalence of AFB1 in food, many strategies should be developed to prevent or remove contamination in order to restore the safety and edibility of food products. Control strategies are divided into pre- and post-harvest techniques. Pre-harvest strategies include the use of genetically altered crops that are resistant to Aspergillus infection. Post-harvest strategies include the use of physical processes or chemical/biological additives to be added to contaminated crops to reduce or transform Aflatoxins such as physical treatment, biological treatment, chemical treatment.

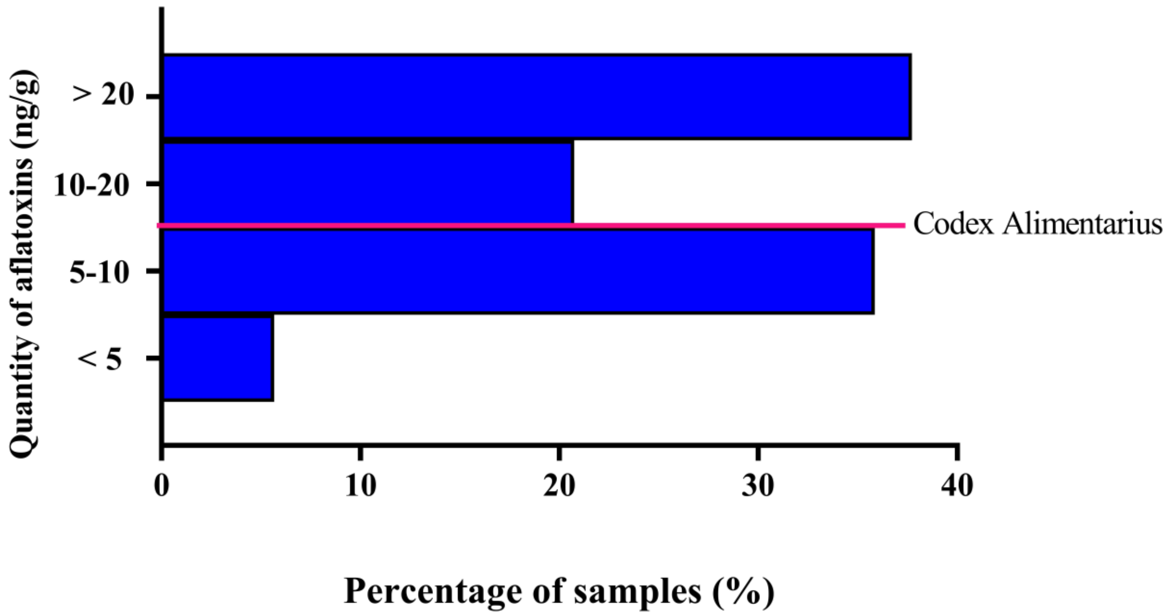

Figure 2. Percentage of maize flour samples contaminated by aflatoxin B_1 in Bobo-Dioulasso (Burkina Faso).

2.4. Association between the average contamination rate by *Aspergillus flavus* and the amount of Aflatoxin B1

In the linear regression, the amounts of aflatoxin B_1 increased with the average contamination rate by *Aspergillus flavus* (Table I). This was expected as *Aspergillus flavus* species have been the subject of several research works that have demonstrated its aflatoxins production capacity (3, 25-27). *Aspergillus flavus* and *Aspergillus parasiticus* are the main species associated with aflatoxins contamination of crops (28).

Table I Results of the regression between *Aspergillus flavus* contamination rates and AFB_1 levels

Classes ng/g	Coefficient [CV]	*pvalue*
≤ 5	20,00[-3.78 ;43.78]	00,097
[5-10[	11,93[-13.66 ;37.51]	0,353
[10-20[	56,06[29.23 ;82.88]	0,000***
>20	64,33[38.83 ;89.93]	0,000***

Conclusion

This study showed that maize flour samples from different markets in Bobo-Dioulasso are highly contaminated with *Aspergillus flavus* and aflatoxins B1. So there are unsuitable for consumption. Future studies would see and orientate the techniques of storage of these flours which could explain the sources of contamination.

Acknowledgments

We wish to acknowledge the assistance of maize flour seller, who agreed to give us maize flour for this study.

References bibliographiques

1. United State Agency International Development. Agriculture and food safety.USAID; 2022. online: https://www.usaid.gov/burkina-faso/agriculture-and-food-security. Accessed February 12, 2023.

2. **Ministère de l'agriculture et des amenagements hydro agricoles Burkina Faso.** Tableau de bord statistique de l'agriculture 2019.2020. online: http://cns.bf/IMG/pdf/tab_bord_agriculture_2019_def.pdf. Accessed march, 12 2023.

3. **Doster M, Cotty P, Michailides T.** Description of a distinctive aflatoxin-producing strain of Aspergillus nomius that produces submerged sclerotia. Mycopathologia. 2009;168(4):193-201.

4. **Commission de la CEDEAO Agence Regional pour l'Agriculture et l'Alimentation A.** Qualité des produits destinés aux produits institutionnels alimentaire: gestions des risques de contamination aux aflatoxines2019:[16 p.]. online: https://www.inter-reseaux.org/ressource/note-gestion-des-risques-de-contamination-des-produits-destines-aux-marches-institutionnels-par-les-aflatoxines/. Accessed April, 16 2023.

5. **Garnett EW.** Mycotoxins in foods and feeds in the United States,. Journal of Animal Science. 1992;70(12,): 3941–9.

6. **European Food Safety Authority.** Opinion of the scientific panel on contaminants in the food chain [CONTAM] related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. The EFSA Journal. 2007;5(3):1 - 127.

7. **Organisation des Nations Unies pour l' alimentation et l'agriculture/ Organisation mondiale de la santé.** Commission du Codex Alimentarius -Manuel de procédure - Vingt-septième édition.FAO/OMS; 2019. online: https://www.fao.org/3/ca2329fr/CA2329FR.pdf. Accessed March, 15 2023.

8. **Cao W, Yu P, Yang K, Cao D.** Aflatoxin B1: metabolism, toxicology, and its involvement in oxidative stress and cancer development. Toxicol Mech Methods. 2022;32(6):395-419.

9. **Gong YY, Cardwell K, Hounsa A, Egal S, Turner PC, Hall AJ, et al.** Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. BMJ (Clinical research ed). 2002;325(7354):20-1.

10. **Cardwell K, Miller JD.** Mycotoxins in foods in Africa. Natural toxins. 1996;4(3):103-7.

11. Lewis L, Onsongo M, Njapau H, Schurz-Rogers H, Luber G, Kieszak S, et al. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. Environmental health perspectives. 2005;113(12):1763-7.

12. Gnonlonfin GJ, Hell K, Adjovi Y, Fandohan P, Koudande DO, Mensah GA, et al. A review on aflatoxin contamination and its implications in the developing world: a sub-Saharan African perspective. Critical reviews in food science and nutrition. 2013;53(4):349-65.

13. Baoua I, Amadou L, Bakoye O, Abdoulaye O, Baributsa D, Murdock L. Maize quality in markets in four West African countries,. Journal of Stored Products Research,. 2016,;69,:26-30,.

14. Kpoda DS, Bandé M, Compaoré AM, Bazié RBS, Meda RN, Somda S, et al. Nutritional, Microbiological, and Toxicological Quality Assessment of Foods Sold in Urban and Suburban Markets in Burkina Faso. Health security. 2022;20(4):298-307.

15. Bandé M, Traoré I, Nikiema F, Méda NR, Kpoda DS, Bazié BSR, et al. Aflatoxins contents determination in some foodstuffs in Burkina Faso and human health risk assessment. Toxicon:X 2022;16(100138).

16. Dean A, Sullivan K, Soe M. Open Source Epidemiologic Statistics for Public Health.2013. online: https://www.openepi.com/Menu/OE_Menu.htm. Accessed,10 March 2023.

17. Barwant M, Lavhate N. Isolation and maintenance of fungal pathogens Aspergillus niger and Aspergillus flavus International Journal of Applied and Natural Sciences (IJANS) 2020; 9(3):47–52

18. Nankai Biotech Co. L. Aflatoxin rapid diagnostic one step Rapid Test Kit online: http://foodrapidtest-com.sell.everychina.com/p-107242453-aflatoxin-rapid-diagnostic-one-step-rapid-test-kit-for-feeds-and-grains.html. Accessed February, 5 2023.

19. Sebők F, Dobolyi C, Zágoni D, Risa A, Krifaton C, Hartman M, et al. Aflatoxigenic Aspergillus flavus and Aspergillus parasiticus strains in Hungarian maize fields. Acta microbiologica et immunologica Hungarica. 2016;63(4):491-502.

20. Masoud W, Kaltoft CH. The effects of yeasts involved in the fermentation of Coffea arabica in East Africa on growth and ochratoxin

A (OTA) production by Aspergillus ochraceus. Int J Food Microbiol. 2006;106(2):229-34.

21. Moradi M, Rohani M, Fani SR, Mosavian MTH, Probst C, Khodaygan P. Biocontrol potential of native yeast strains against Aspergillus flavus and aflatoxin production in pistachio. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2020;37(11):1963-73.

22. Ware LY, Durand N, Nikiema PA, Alter P, Fontana A, Montet D, et al. Occurrence of mycotoxins in commercial infant formulas locally produced in Ouagadougou (Burkina Faso) Food Control. March 2017;73:518-23.

23. Warth B, Parich A, Atehnkeng J, Bandyopadhyay R, Schuhmacher R, Sulyok M, et al. Quantitation of mycotoxins in food and feed from Burkina Faso and Mozambique using a modern LC-MS/MS multitoxin method. Journal of agricultural and food chemistry. 2012;60(36):9352-63.

24. Ministère de l'agriculture et des amenagements hydro-agricoles. Bilan alimentaire du Burkina Faso 2013-2014.2020. online: https://www.agriculture.bf/upload/docs/application/pdf/2020-07/burkina_faso_bilans_alimentaires Accessed March, 15 2023.

25. Amaike S, Keller NP. Aspergillus flavus. Annual review of phytopathology. 2011;49(1):107-33.

26. Johnsson P, Lindblad M, Thim A, Jonsson N, Vargas E, Medeiros N, et al. Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts. World Mycotoxin Journal. 2008;1 (2):127-37.

27. Ito Y, Peterson S, Wicklow D, Goto T. Aspergillus pseudotamarii, a new aflatoxin producing species in Aspergillus section Flavi. Mycological Research. 2001,;Volume 105(2):233-9.

28. Frisvad J, Hubka V, Ezekiel C, Hong S, Nováková A, Chen A, et al. Taxonomy of Aspergillus section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. Studies in mycology. 2019;93(1):1-63.