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Contribution to the Study of Fungal Strains Contaminating Peanut Pastes in Bangui (Central African Republic)

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ABSTRACT

Introduction: Peanut pastes are food products resulting from artisanal or industrial processing, used in cooking in Africa in general and in Central African Republic in particular. These peanut pastes are often contaminated by molds and filamentous fungi involved in the degradation of hygienic and organoleptic or even toxicological quality. This study aims to determine the epidemiological profile of molds contaminating peanut pastes sold on the Central African market. Methodology: This was a cross-sectional study carried out from June to September 2023. Samples of peanut pastes sold on Central African market were taken and analyzed at the National Laboratory of Clinical Biology and Public Health using the conventional microbiology method according to ISO 7954 standards. The data obtained were collected in the ODK 2023.3.1 application and analyzed with the Epi Info 7 software. A multivariate analysis by logistic regression, Ficher's exact test, and chi² at the 5% threshold ($p < 0.05$) were used. Results: A total of 320 samples were taken in the main markets of the city of Bangui. The overall prevalence of contamination of peanut paste samples was 60%. The proportion of contamination per market was 95% at the Petevo market, 65% at the Sango market, 60% at the Miskine and Combattant markets, 55% in the Boy-rabe and Centrale markets, 50% at the Mamadou Mbaïki market and 40% at the market Ouango. The count of the total fungal flora and 10.85×10^6 CFU/g at the Boy Rabe market; 16.45×10^6 CFU/g at Combattant market; 10.3×10^6 CFU/g at the Central market, 12.8×10^6 CFU/g at the Miskine market; 10.2×10^6 CFU/g at the Mamadou Mbaïki market; 10.8×10^6 CFU/g at the Ouango market; 18.05×10^6 CFU/g at the Petevo market and 13.65×10^6

CFU/g at the Sango market. The prevalence of contamination by different fungal species was 1.88% of the species *Penicillium* sp.; 11.25% of *Mucor* sp.; 10.63% of *Aspergillus terrei*; 3.13% of *Aspergillus niger*; 1.25% of *Aspergillus medullans*; 28.13% of *Aspergillus flavus*; 2.50% of *Aspergillus fumigatus*. Peanut pastes stored beyond three days were more contaminated (94.19%). Conclusion: The results of this study made it possible to highlight strains of mold that impact the hygienic and organoleptic quality of peanut pastes sold at the Central African market. Most of the isolated strains were the *Aspergillus flavus* species which is recognized by its toxigenic effects. This species is much more incriminated in the contamination of foodstuffs with the production of the toxin which causes underlying pulmonary pathologies in humans.

1. INTRODUCTION

Peanut (*Arachis hypogaea*) is a plant of the legume family in which the central stem produces flowers. The fruit is a pod containing one to three seeds that develop in branches below the ground. Peanut seeds are used to make oils often used for preparing food in kitchens. Peanut seeds are also eaten raw or roasted [1]. They contain chemical substances (heavy metals, bacterial toxins, aliphatic hydrocarbons, pesticides, etc.) or pathogenic biological agents (viruses, bacteria fungi, and molds) [2, 3]. Molds, mycetes, or even filamentous fungi are parasites of the fungal kingdom coming from the *zygomycota* and *Ascomycota* branches with more than 1000 species. They are important players in the microbial world involved in a multitude of biological processes with economic interest. Certain Ascomycetes are used in the food industry (involved in the fermentation process) for the manufacture of food products. Others secrete toxins that could alter the hygienic and organoleptic quality of food products [4]. These microbial or toxic effects can impact the health of humans and animals [5]. According to the Food and Agriculture Organization of the United Nations, approximately 25% of global food crops are contaminated by mold [6, 7]. The *Aspergillus* genus among other fungal species is much more incriminated in the contamination of foodstuffs with the production of the toxin and causes underlying pulmonary pathologies in humans [8]. Humans are exposed by ingesting contaminated food. Beyond the food route, humans could also be contaminated through the respiratory route (inhalation) with *Aspergillus flavus* conidia. These conidia constitute one of the main allergenic agents of bronchial aspergillosis in humans and are responsible for pulmonary infections in immunocompromised patients [6]. In the fields or during storage in silos or granaries, certain strains of the *Aspergillus* genus produce aflatoxins which are secondary metabolites recognized for their carcinogenic, immunosuppressive, and teratogenic roles. The species *Aspergillus flavus*, parasiticus, and nomius are the best known and have been the subject of several research studies that have demonstrated their capacity for producing aflatoxins. In addition to these three species, aflatoxin production capacities have been discovered more recently in the species of *Aspergillus tamarii,* ochraceoroseus, and pseudotamarii [1]. Peanut paste, by its biochemical composition and nutritional value, is potentially favorable to microbial growth and in particular mold. The consumption of peanut paste is common throughout the world, in Africa and Central African Republic in particular. Artisanal processing, means of preservation, transport, and places of sale can encourage the development of mold [7, 9]. Peanut cultivation is one of the most cultivated agricultural products in Central African Republic due to its socio-economic and medicinal interest and the crucial role it plays in Central African cuisine as in other countries in the sub-region. Thus, few studies have been carried out in this area of contamination of peanut pastes by mold in Central African Republic although it is one of the most popular ingredients in Central African cuisine. It is in this context that this study was carried out, the objective of which was to characterize the molds contaminating peanut pastes sold at the Central African market.

2. MATERIAL AND METHODS

2.1. Biological Material

The biological material consisted of peanut paste which is used as a condiment in the preparation of sauces popular with the Central African population (Table 1).

2.2. Method

2.2.1. Sampling

A total of 320 samples of peanut pastes sold in the eight (8) main markets in the districts of the city of Bangui were collected. The samples were taken by random method from the sellers from October to November 2023 in aseptic conditions and sent to the laboratory for microbiological analyses.

2.2.2. Field Investigation

Interview-type questionnaires structured and tested beforehand were sent to the sales people. Information on production technology, conservation, and the assessment of the level of knowledge of the sellers on the concepts of food safety was recorded in the questionnaire. The collected data was recorded in the ODK collection v2023.3.1 application.

2.2.3. Microbiological Analysis

- **Mold count**

The total fungal flora was isolated on agar medium with chloramphenicol [10]. And counted according to the recommendations of the ISO 7954 standard [11]. 25 g of each sample was added to 225 mL of buffered peptone water to obtain the stock solution. A decimal dilution was made from the stock solution. Two Petri dishes containing Sabouraud Chloramphenicol medium were inoculated using the mass technique, *i.e.* 1 mL of the two successive dilutions was placed at the bottom of two Petri dishes, and 8 mL of Sabouraud chloramphenicol medium was poured onto 1 mL of the two dilutions. in the two Petri dishes. The two Petri dishes were rotated on the bench and left at room temperature to solidify. The Petri dishes were incubated at 30˚C for 3 to 5 days. Viable colonies were developed on the surface of the culture medium as a large colony (brown and wrinkled in color). Colony enumeration was done from 24 to 72 hours. The detection limit of a fungal species under the experimental conditions used is 10 CFU/g of sample [12].

Table 1. Composition of 100 g of peanut seed (FAO, 2017).

Constituents	Raw with skin (%)	Raw without skin(%)
Water	5.66	5.4
Proteines	26	26.3
Fat	47.5	48.4
Carbohydrates	18.6	17.6
Fibers	2.4	1.9
Ashes	2.3	2.3
Minerals	1.15	1.15
Others	0.5	0.5
Energy (J)	2.361	2.378

- **Isolation and identification of fungal strains**

The stock solution was then inoculated by flooding on the surface of the agar and incubated in the incubator for 24 hours to 72 hours to obtain colonies or supposedly pure strains [8, 13]. The previously isolated colonies were subcultured successively until pure strains were obtained [14]. These pure strains obtained were subjected to morphological identification carried out by observation. Identification is based on macroscopic and microscopic classification criteria [15]. The strains were also identified by their cultural characteristics, the growth speed (diameter of the colonies), the color of the colonies, the presence or absence of metules (seriation); the arrangement and shape of the phialides, the color of the conidiophore and conidia [16, 17]. The pure strains obtained were then preserved on a Czapek Yeast Extract Agar medium inclined in cryovials at +4˚C [18].

- **Macroscopic appearance**

The morphological criteria are based on the macroscopic appearance of the colonies on the agar by observation of their color on the front and back, their relief, their appearance (filamentous, sticky, etc.), their transparency (opaque, translucent), the appearance of the contours and the Pigmentation sometimes diffusing into the agar. Also as highlighted above, the growth speed, the odor released, and the texture of the colonies [19].

- **Microscopic appearance**

The fragment of the pure strain (a few spores and a mycelial fragment at the margin of the thallus) was observed by two techniques for confirmation. The colonies are collected with a sterile platinum loop at the edge of the colony containing the conidio genic structures transferred to a slide, to which the drop of methylene blue has been added as a dye from the bottom of the slide. Microscopic observation was made at X10 and X40 magnifications [16, 20] to observe the mode of grouping of conidia (spores) and their structures. The second technique involved lightly placing a piece of transparent tape on the surface of the culture and at the edge of the colony. This ribbon was stuck on a slide and observed microscopically at X100 and X400 magnification. Molds are identified based on their morphological characteristics and the appearance of the colonies [8].

2.2.4. Statistical Analysis of Data

The data was collected using the ODK collection software. The Epi info version 6 software was also used for data analysis and processing. Descriptive statistical tests were used to calculate central tendencies. With $p < 0.05$ two-sided, the test was considered statistically significant with a 95% confidence interval. Some results were expressed as frequency and percentage, and SPS software was used for some trend analyses.

3. RESULTS

The answers to the quiz made it possible to understand the manufacturing (**Figure 1**) process from the choice of raw materials, the conservation processes and the minimum and maximum duration.

3.1. Manufacturing Diagram

The production of peanut paste involves different stages such as shelling, fire roasting, blanching of

Figure 1. Peanut paste manufacturing diagram.

the seeds, and grinding. The other red film must be removed before grinding, possibly by hand.

The microbiological analysis carried out on Sabouraud chloramphenicol agar made it possible to identify the different fungal strains based on their cultural and microscopic characteristics based on the *aspergillus* head of the genus *Aspergillus.*

3.2. Aspect of Different Fungal Colonies on Sabouraud Chloramphénicol Agar and Microscopy

Figure 2 shows the strains of *Aspergillus flavus*, *fumigatus* and *niger* on the different sabouraud media and a microscopic view of the strains of *Penicillium, Mucor* spp. and *Aspergillus.*

3.3. Prevalence of Overall Contamination of Peanut Pastes by Molds

60% of pasta is contaminated compared to 40% of uncontaminated pasta (Figure 3).

3.4. Prevalence of Mold Contamination of Peanut Pastes by Market

The Petevo, Sango, Miskine, and Combattant markets had a higher prevalence of mold contamination of peanut pastes than the other markets (Figure 4).

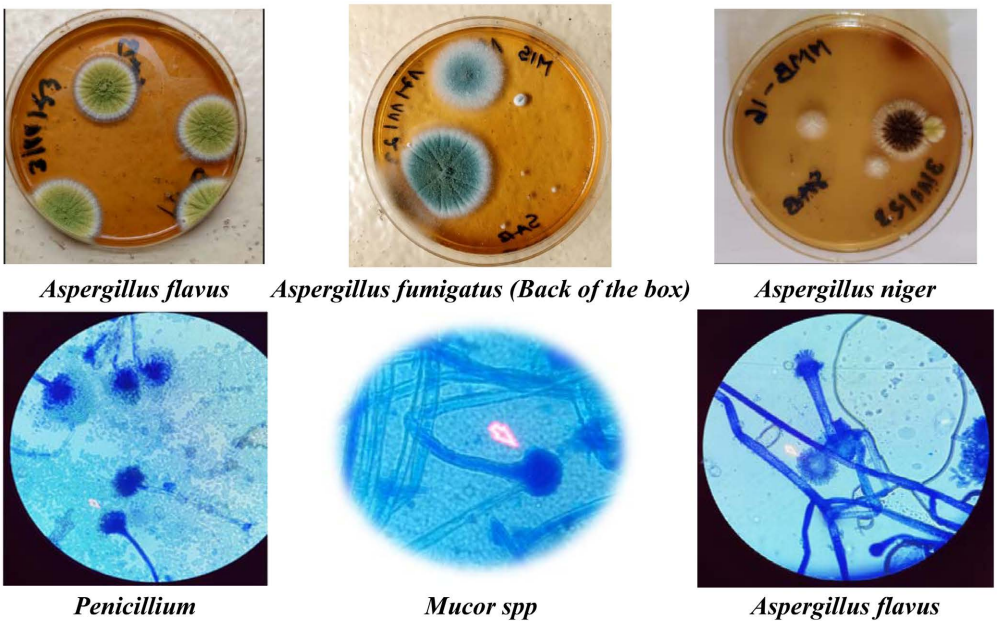

Figure 2. Different types of colony structures in Sabouraud Chloramphenicol culture media.

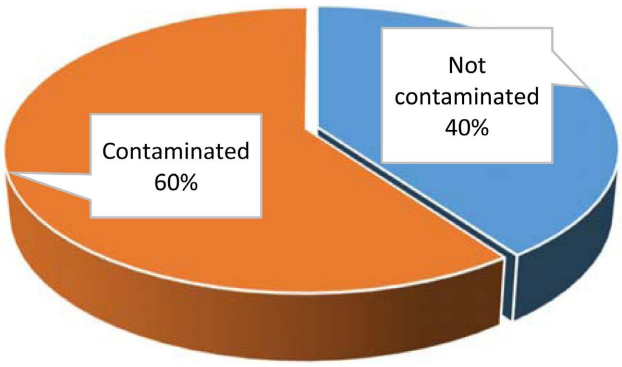

Figure 3. Overall proportion of contamination of peanut pastes by mold.

3.5. Counting of Molds Contaminating Peanut Pastes by Market

The total fungal flora was higher in the Petevo, Combattant, and Sango markets than in the other markets (Table 2).

3.6. Overall Prevalence of Fungal Species Contaminating Peanut Pastes

The genus *Aspergillus flavus* represents 28.13% of the contamination compared to other isolated species.

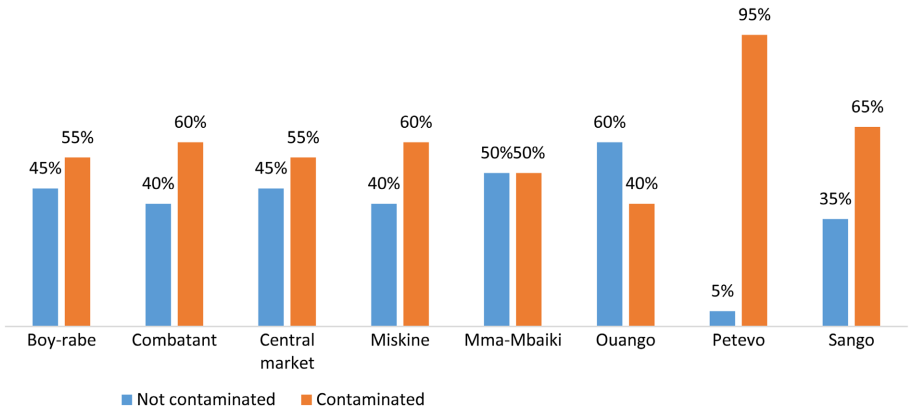

Figure 4. Proportion of contamination of peanut pastes by mold by market.

Table 2. Count of total fungal flora in peanut pastes by market.

	Market							
	Boy-rabe	Combattant	Centrale	Miskine	Mamadou Mbaïki	Ouango	Petevo	Sango
Fungal load UFC/g	10.85×10^6	16.45×10^6	10.3×10^6	12.8×10^6	10.2×10^6	10.8×10^6	18.05×10^6	13.65×10^6

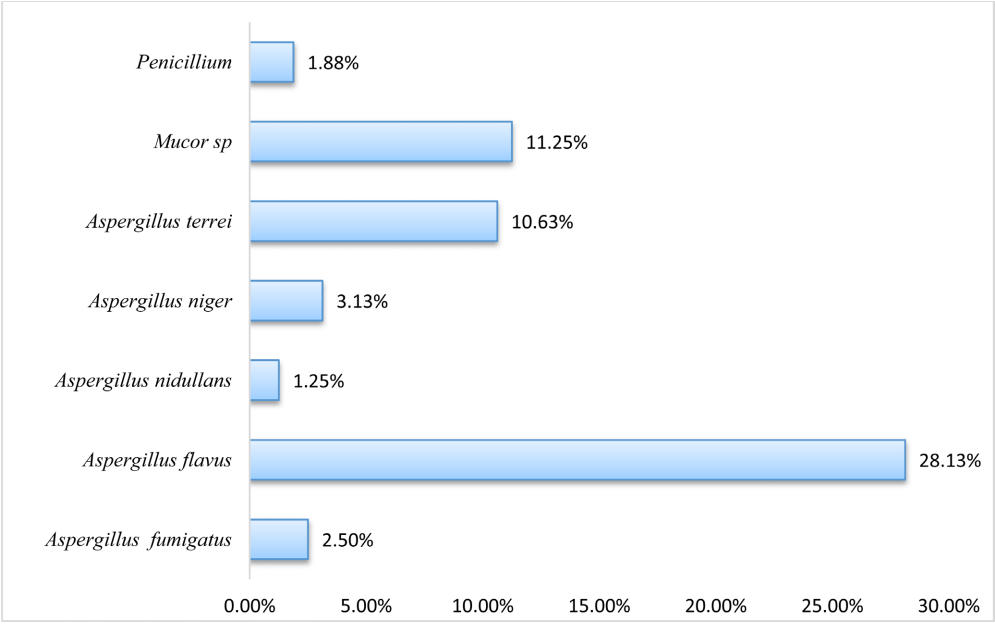

Figure 5. Proportion of contamination of peanut pastes by different fungal species.

Aspergillus flavus represent 28.13%, *Penicillium* spp. 1.88% and *Aspergillus nidullans* 1.25% of all isolated species (Figure 5).

3.7. Prevalence of Mold Contamination of Peanut Pastes by Market

The species *Aspergillus flavus* was isolated in almost all markets and the species *Aspergillus nidullans* was only found in the Ouango market (Table 3).

3.8. Proportion of Contamination of Peanut Pastes by Molds Depending on the Shelf Life

Pastes that were kept for more than three days were more contaminated than those kept for less than three days (Figure 6).

4. DISCUSSION

Samples of peanut pastes sold in the main markets of the eight districts of the city of Bangui were taken by the random method and analyzed. The data obtained made it possible to highlight the profile of molds contaminating peanut pastes in the Central African Republic. The technique used was that of conventional microbiology according to ISO 7954 standards about the isolation and enumeration of

Table 3. Proportion of contamination of peanut pastes by fungal species by market.

Market	*Aspergillus fumigatus*	*Aspergillus flavus*	*Aspergillus nidullans*	*Aspergillus niger*	*Aspergillus terrei*	*Mucor* sp.	*Penicillium*
Boy-rabe	50.00%	4.44%	0.00%	60.00%	5.88%	11.11%	33.33%
Combatant	25.00%	11.11%	0.00%	20.00%	5.88%	16.67%	33.33%
Marché centrale	0.00%	17.78%	0.00%	0.00%	11.76%	5.56%	0.00%
Miskine	25.00%	11.11%	0.00%	20.00%	5.88%	16.67%	33.33%
Mma-Mbaiki	0.00%	17.78%	0.00%	0.00%	0.00%	0.00%	0.00%
Ouango	0.00%	6.67%	100.00%	0.00%	11.76%	5.56%	0.00%
Petevo	0.00%	31.11%	0.00%	0.00%	17.65%	11.11%	0.00%
Sango	0.00%	0.00%	0.00%	0.00%	41.18%	33.33%	0.00%

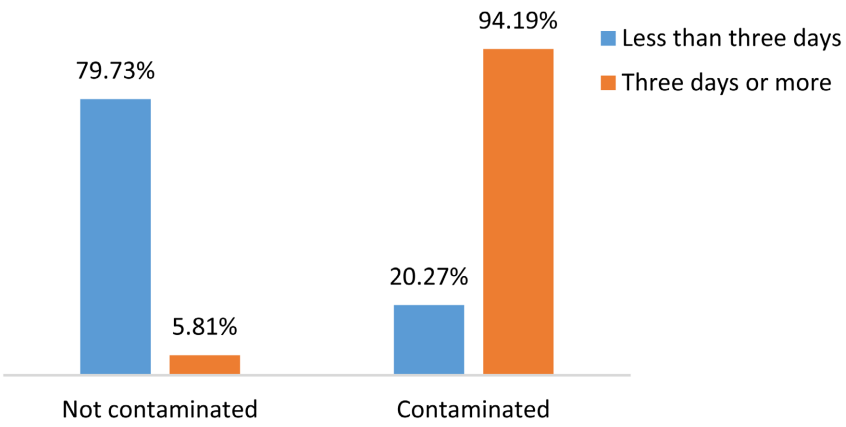

Figure 6. Contamination of peanut pastes depending on storage time.

microorganisms in food samples [11-13] and identification based on macroscopic characters, microscopic and cultural studies of the different molds [19]. The overall prevalence of mold contamination of peanut pastes was 60% (**Figure 3**). This result is in line with those obtained by Sangaré *et al.* in 2021 [20] in a study carried out in Burkina Faso which highlighted a high prevalence of mold in pastes made from peanuts (*Arachis hypogaea*), corn (Zea mays), Sesame (*Sesamum indicum*) and Moringa (*Moringa oleifera*). These results also corroborate the literature which reveals the presence of mold most often in certain food groups such as peanut butter [21]. The distribution of contamination by fungal species is of the order of 28.13% of *Aspergillus flavus*, 11.25% of *Mucor spp*, 10.65% of *Aspergillus terrier*, 3.13% of *Aspergillus niger*, 2.5% of *Aspergillus fumigatus*, 1.25% of *Aspergillus nidulans* and a small proportion of 1.88% of *Penicillium spp* (**Figure 5**). The distribution of species by market is 50% of *Aspergillus fumigatus* at the Boy-rab and Combatant market, the proportion of *Aspergillus flavus* is 31% at the Petevo market, 100% at of *Aspergillus nidullans* at the Ouango market ,*Aspergillus niger* is 60% in the boy rab market and *Penicillium spp* is 33,33% in the Boy-rab, fighter and miskine market (**Table 3**). These results corroborate with the results of some studies carried out by Mahideb *et al.* in 2015 [22]; Rebbouh *et al.* in 2009 who revealed contamination of peanut pastes by strains of *Aspergillus* (33%) and *Mucor* in the order of 10% [15, 23]. The high rate of the genus *Aspergillus* could be explained by the fact that this strain is often found in the soil and the air through its spores, therefore, the contamination of these foods exposed to the open air during their sale can be easily contaminated by these molds. Scheidegger *et al.* explained that molds present in nature would colonize agricultural products, their presence on the surfaces of the seeds was assimilated to poor storage conditions which could explain their development in peanut pastes [17]. Just as Zongo *et al.* in a study carried out in Chad confirms that peanut pastes are food products from the cereal family and are favorable to the growth of molds [23]. The *Penicillium* species is present in our study with a proportion of 1.88%. This result is in line with that of a study carried out by D'Mello *et al.* in 1998 which confirms the fact that species of the *Penicillium* genus develop much more at low temperatures, which would explain the high rate of contamination of pasty products and Ribal *et al.* in temperate regions [24, 25]. The enumeration of the total fungal flora on the Sabouraud Chloramphenicol medium gave a high load of molds in the Pétévo, Sango, Miskine, and Combattant markets (**Table 2**). The potatoes sold at the Ouango market were less contaminated (40%). The contamination of these pastes was high at 95% in the Pétévo market (**Figure 4**). Our results are similar to those of a study carried out by Bennoudia *et al.* in 2016 in Algeria which revealed in peanut pastes a high value above the detection limit of the total fungal flora which is 10 CFU/g in peanut pastes [8, 12]. On the other hand, our results differ from those of a study carried out by Hanak *et al.* who found contamination of non-disinfected grains by external mycoflora of 71.59% and of grains disinfected by deep mycoflora of 15.82% [26]. The prevalence of contamination of peanut pastes by mold by market showed a low proportion of contamination at the Ouango market unlike that of other markets. This difference could be explained by the fact that in the Ouango market, the sellers are exclusively peanut paste sellers. In other markets, sellers combined the sale of peanut pastes with other food products such as smoked fish. The results of this study are consistent with those of a study carried out by Hissein *et al.* which revealed contamination of food products by mold due to non-compliance with good manufacturing practices for peanut pastes, lack of respect for hygiene and conservation rules (**Figure 6**) [27].

5. STUDY LIMIT

The choice of markets for our sampling was not representative of all markets in the Central African Republic. Few studies have been carried out in this area, which has made it difficult to discuss our results.

6. CONCLUSION

The results of this study made it possible to highlight strains of mold that have a negative impact on the hygienic and organoleptic quality of peanut pastes sold on the Central African market. Most of the strains isolated from peanut pastes were of the *Aspergillus flavus* species. This species is known for its tox-

igenic effects. The artisanal processing, means of preservation, transport, and places of sale of peanut pastes can encourage the development of mold. *Aspergillus flavus* is responsible for bronchial aspergillosis in humans, which is a pulmonary infection in patients. Consequently, the high rate of these molds in peanut pastes would be a danger to consumers.

CONFLICTS OF INTEREST

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

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