

Identification of Molds Strains Associated with Some Cereals and Oils Seeds and Their By-products in Selected Municipalities of Côte d'Ivoire

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Abstract The presence of mycotoxins in some foods for human consumption is emerging as an important public health issue and has created a need for more information about the occurrence of molds in tropical crops and by-products. This study aimed to investigate the abundance and diversity of total microfungi in 176 samples of oilseeds/derived pastes, maize grains/flours, and millet collected from Abobo markets and Adjamé two towns in Abidjan. To isolate contaminating molds, groundnut, and pistachio seeds, maize, and millet grains were cultured directly on Sabouraud chloramphenicol agar medium while pastes from oil seeds or flours from cereals were analyzed using the decimal dilution method. The isolates of microfungi were purified by cloning subculture technic. The macroscopic and microscopic morphological characteristics of each mold isolate were carried out for the identification of its genus. A total of 17 isolates of molds were found in oilseeds and their pastes and 36 from cereals and their derived flours. Based on their characteristics, these fungi isolates were grouped into 9 genera: *Mucor*, *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizomucor*, *Rhizopus*, and *Chrysosporium*. Among them, the mold strains belonging to the *Aspergillus* genus predominated the mycoflora with isolation frequency ranging from 75 to 100 %. The predominance of the *Aspergillus* fungi highlighted a serious risk of mycotoxins production in the crops and their by-products for Ivorian consumption. Proper storage is recommended owing to the public health concern due to oilseeds, cereals, and their by-product shelf life, biostability, and safety.

Keywords: *identification molds genera, cereals, oilseeds, by-products*

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1. Introduction

Groundnut, pistachio, maize, and millet are widely produced and consumed foods around the world [1-6]. Groundnuts and pistachio are known for their oil which is rich in nutrients such as lipids, proteins, and mineral salts [7,8]. In Côte d'Ivoire, these oilseeds are intended for domestic consumption in the form of groundnut and pistachio seeds paste as well as for trade [9].

Cereals, particularly maize and millet, and their derived products play an important nutritional, social, and economic role. For many developing countries, cereals represent the mainstay of the diet of generally low-income rural populations. The most frequently used cereals are corn, sorghum, and millet [10,11]. Maize and millet in particular are generally consumed in the form of semolina,

flour, and grains. The introduction of solid foods as diversification and complementary food in children is a method traditionally used to complement breastfeeding and gradual weaning before the introduction of the family meal. This diversification is deeply influenced by family food cultures and the socio-economic environment of the infant's development and growth [12].

Maize and millet-based foods for children must be safe and not contain pathogenic germs or toxins, even less toxic chemical residues likely to affect the infant's health. According to the Codex Stan 74-1981 standard of the Codex Alimentarius, infant flours must be prepared, packaged, and stored under conditions compatible with hygiene [13]. They should comply with the provisions of the code of hygienic practice for infants and children. This code gives microbiological specifications of an advisory nature, different depending on whether it is cooking flour or instant flour. The diets of infants and young children

are more restricted, therefore the significance and potential health risk of any contaminant in foods consumed by infants are heightened and particular attention should be given. However, the production and marketing of cereal flour are mainly done in the informal and sometimes uncontrolled sector by small producers using an artisanal or semi-artisanal process in conditions of insufficient sanitation [12,14].

Due to their biochemical composition, oilseeds such as groundnut and “African pistachio” as well as maize and millet are particularly sensitive to microbial contamination, in particular by filamentous fungi. Fungal contamination of these commodities is a chronic problem that results in huge losses in quality, quantity, and nutritional value. Molds reduce the quality of groundnut seeds, pistachio seeds, corn grains, millet, and their derived products by producing mycotoxins. The fungal contaminations should not be underestimated because they can be severe and sometimes lead to serious pathologies that are commonly fatal. Indeed, several studies have revealed that mycotoxins have various toxic effects, particularly on the esophagus, liver, kidneys, and immune system, and cause deformations in the embryo [15,16,17]. This situation is particularly because mycotoxins are generally and particularly thermostable. Therefore, they can be transferred to food, even after microbial stabilization steps, such as heating and extrusion [18,19]. At the same time, it was noted that very few studies have been conducted on the diversity of mold strains contaminating these oilseeds, cereals, and their derivatives. It is true that [9] isolated several species of molds from peanut butter marketed in Abidjan (Côte d’Ivoire). However, few studies have been conducted on the identification of the molds contaminating African pistachio, maize, millet, and by-products produced in Côte d’Ivoire. So, this study was initiated to identify the genus of molds contaminating groundnut, “African pistachio”, maize, millet, and their derivatives.

2. Materiel and Methods

2.1. Material

The biological material used in this study consisted of maize grains, dry millet, dry and raw bulk groundnut seeds, dry and raw pistachio pips, and their derived products under artisanal conditions and collected in at least three markets.

2.2. Sampling

Sampling was carried out in three markets per city of Abobo and Adjamé. This choice was guided by the fact of the availability of the plant material of interest, the high density of populations in different age groups from low-income households sourcing, one hand, corn, millet, and their derived products to feed their young children. On the other hand, these populations are large consumers of dry groundnut seeds, dry pistachio pips, and their derived products (groundnut and pistachio paste as well as *lidèguè*: salted, sweet, and spicy groundnut paste). Maize and millet grains and their derived products were sampled every two weeks in each of the markets during August and September 2021. In total, ninety-six (96) samples of 100 g around each including 24 samples of maize grains, millet grains, maize flour, and millet flour. About 300 g of samples per product were purchased from sellers, placed in foot bags, and stored in a cooler with dry ice. The samples of groundnut seeds, raw pistachio pips, and their derived products were collected in plastic bags from four (4) producers in the markets at the rate of two producers per commune. Two passages were carried out at each seller with 2 repetitions for each sample to be analyzed. A total of 80 samples were taken in the two municipalities. Once in the laboratory, the collected samples were stored in the refrigerator at a temperature of approximately 4°C for no more than 48 hours.

3. Methods

3.1. Isolation of Molds Strains

3.1.1. From Cereals Grains and Oilseeds

The isolation of molds contaminating maize grains, millet, groundnut seeds, and pistachio seeds was carried out by the technique of direct cultivation of grains, and seeds with Sabouraud chloramphenicol. However, for maize and millet grains, 5 grains of each cereal were soaked for one minute in a 1 % sodium hypochlorite solution [2,20]. The grains thus washed were rinsed with distilled water and then drained in paper towels. Finally, they were carefully cultured on a Sabouraud medium with chloramphenicol under aseptic conditions.

The inoculated Petri dishes were incubated at 30°C and examined daily for 4 to 5 days. The cultures produced were locked up in a closed enclosure at ambient temperature for 4 to 5 days.



Figure 1. Photographs of some samples: (1) groundnut seeds, (2) groundnut paste, (3) *lidèguè*, (4) pistachio seeds, (5) pistachio paste

3.1.2. From Cereal Flours and Oilseeds Pastes

For the isolation of molds contaminating maize and millet flours, 10 g of flours were introduced aseptically into a sterile stomacher bag. Then, 90 mL of physiological water was added thereto. The whole was homogenized by mixing for 3 to 5 minutes. The suspension obtained constituted the stock suspension and corresponded to the 10^{-1} dilution. The stock suspension was kept at ambient laboratory temperature for 30 minutes to revive the microorganisms present. Successive decimal dilutions up to 10^{-9} were made from the stock suspension. For each decimal dilution, 0.1 mL was inoculated by spreading on Sabouraud medium with chloramphenicol and then incubated at 30°C for 3 to 5 days. Three Petri dishes per dilution were inoculated and incubated. For products derived from groundnut and pistachios, 5 g of each type of paste were suspended in 45 mL of sterile physiological water to which 2 drops of tween 80 were added. A series of dilutions ($10^{-1}/10^{-2}/10^{-3}$) was carried out from the stock solution and thus deducted. The goal is to reduce the load and have isolated individuals to be able to purify them. Subsequently, 1 mL of a load of each dilution was deposited and inoculated in a rake on the surface of the Sabouraud medium with chloramphenicol.

3.2. Purification of Molds Strains

The pure fungal isolates were obtained after several subcultures on Sabouraud chloramphenicol agar of the isolates. The technique consists of taking a small mycelial and cutting it into a single point in the center of the agar using a sterile Pasteur pipette. The different cultures were incubated at 30°C for 4 to 6 days. The fungal vegetative apparatuses obtained were differentiated after macroscopic observation with the naked eye, taking into account the phenotypic characterization.

The boxes from isolation included several vegetative devices with different looks, colors, and textures. In the event of contamination by another fungal strain, the strains were purified by subculturing a terminal hypha in the center of the box containing the same medium and under the same incubation conditions until strains were obtained pure [2,20].

3.3. Study of the Cultural Characteristics of Molds Isolates

The study of the macroscopic morphological characters was made by observation with the naked eye of the surface and the back of the pure fungal strains obtained. The characters studied were the mycelium (color and texture of the thallus, color of the underside of the vegetative apparatus contour of the vegetative apparatus, and speed of apical growth), spores (density on the thallus, aspect of the spores: granular or powder), the uniformity of spore color, the presence of diffusible pigments and exudates [2,20].

3.4. Study of Morphological Characteristics

To carry out the morphological identification isolates, fresh smears were made from fragments of pure cultures

between the slide and coverslip in the presence of a drop blue (Botton *et al.*, 1990). Microscopic observation of the smears was carried out at X40 magnification and if necessary X100. The observation concerned the filament (absence or presence of partitions, presence or absence of branching), the sporiferous organs (structure, typical organization, etc), the spores (shape, color, the texture of the walls, the mode of grouping in chains, differentiation into thallospores, etc). The identification was carried out based on taxonomic schemes according to morphological characters [2,20].

3.5. Estimation of Fungal Contamination Frequencies

The frequency and relative density of genus and species contaminating maize and millet grains, and flours were calculated according to the formulas of Marasas *et al.* (1998):

$$\text{Frequency}(100) = \frac{\text{Number of samples contaminated by a genus}}{\text{Total number of samples}} \times 100$$

$$\text{Relative density}(100) = \frac{\text{Number of isolates of genus}}{\text{Total number of isolated genera}} \times 100$$

4. Statistical Analyzes

The results from the analysis of maize grains, millet, and their derivative products were analyzed with the XLSTAT software version 2021. Once-way analyzes of variance (ANOVA) were carried out to determine whether there are significant differences according to the test Fisher the threshold of 5%. Contamination levels and isolation frequencies of isolates in oilseeds and derived products were assessed using Excel software.

$$\text{Contamination level}(\%) = 100 \times \frac{n}{N}$$

n : number of matrices contaminated by the isolate
 N : total number of matrices studied

$$\text{Frequency}(\%) = 100 \times \frac{t}{N}$$

t : number of times detected for an isolate
 N : total number of fungal isolates detected.

5. Results

5.1. Characteristics of Molds Isolates

After the isolation of the fungal colonies, their cultural and microscopic identifications were carried out according to the determination keys and picked up by recent studies [2,20]. The results showed 17 isolates in oilseeds and their derivatives, while 36 isolates were contaminated cereals and their derivatives. All these molds have been grouped into nine (9) genera: *Mucor*, *Aspergillus*, section *flavi*,

Aspergillus section nigri, *Aspergillus fumigati*, *Penicillium*, *Fusarium*, *Rhizomucor*, *Chrysosporium*, and *Rhizopus*. However, *Aspergillus fumigati* has only been identified in cereals and their derived products. The genus *Aspergillus* was dominant.

5.1.1. Molds Belonging to the Genus *Mucor*

Regarding the genus *Mucor*, two species have been identified in groundnut seeds, pistachio seeds, and their derived products. At the cultural level, the first species is characterized by a very invasive vegetative form with a high growth rate. With a cottony appearance, its relief is domed. On the reverse of the dish, the culture shows no production of pigment or exudate. At the microscopic level, the filaments of this isolate are not septate or branched. The sporangiophore bears a globose vesicle and a sporangium containing conidia of variable shape and size and brown. As for the second species, it appears to be less invasive with an average growth in the front of the Petri dish, the culture looks like wool and is gray. On the reverse side of the culture, there is an orange-yellow coloration due to the production of pigment. The filaments of this isolate are not septate nor branched but voluminous. Its sporangiophores are long and end in a small columella. Each bears a sporangiophore containing sporangia. The sporangia are very small in size.

The species of *Mucor* observed only in cereals and their derivatives show very rapid growth, a cottony colony resembling discolored hair very frizzy, circular, brown in color, and raised relief on Sabouraud chloramphenicol. The back of the box shows exudates. Microscopic observation revealed that the filaments are septate and branched. The sporiferous organ is a sporangiophore. The conidia are homogenous, globose, and smooth.

5.1.2. Molds of the Genus *Aspergillus*

5.1.2.1. *Aspergillus* Section Flavi

Four species of *Aspergillus* section flavi have been detected in oilseeds and their derived products. The culture of the first species is grainy in appearance and varies in color centripetal from white to golden yellow.

The contour of the culture is irregular and its relief is flat. Its growth rate was average. The reverse side of the culture is colorless and therefore does not produce pigment. The filaments of this isolate are septate but unbranched. Its conidiophores are very long and end in large globular vesicle-bearing metulae the phialides organized in a biseriata structure. The exo-conidia produced are round in shape with echinulate walls, of variable size but dominated by medium-sized spores. The second isolate presents a centrifugally green, yellow to white powder to fluffy appearance on the front. Its culture has an average growth on Sabouraud chloramphenicol. Its border is imperfect and the relief is flat with a domed center. Culture produces neither pigments nor exudates. Its filaments are septate and unbranched. The very elongated conidiophores carry phialides inserted directly on a spherical vesicle and organized in a uniseriate structure. The exo-conidia are globose or subglobose in shape and pale green in color. The third species of section flavi identified in oilseeds and their products showed a concentric culture on the surface of the dish with three circles of dark green greenish yellow, and white color in a centrifugal fashion. This culture has a powdery appearance, with a flat relief and average growth on the agar. There is no pigment or exudate on the back of the box. The filaments of this mold are septate and branched. The conidiophores end in a large elongated vesicle bearing mutilate then phialides organized in a biseriata structure. The exo-conidia produced are green in color, of variable shapes (elongated, round) and sizes (small, medium, and large) but dominated by medium-sized spores. The last isolate from the flavi section of oilseeds is minimally invasive with an irregular outline. This mold has white, yellow to green colors. It is a perfectly round crop. The reverse without pigmentation appears in the form of a cloud. Its filaments are unchambered and unbranched. The sporiferous organ of this strain is a conidiophore bearing a small vesicle. The phialides are inserted directly into the vesicle, a structure organized as a biseriata head. The ex-conidia grouped in chains isolated are of variable shapes and sizes as well as pale green in color.



Figure 2. Microscopic cultural and morphological characteristics of molds genus of *Mucor* (A: microscopic cultural; B: morphological characteristics)



Figure 3. Microscopic cultural and morphological characteristics of molds genus of *Aspergillus* section flavi (A: macroscopic characteristics; B: microscopic characteristics)

Two species of *Aspergillus* were identified in cereals and their derivatives. The first shows colonies of medium-growth, an island in the form of islands of brown color, and flat relief on Sabouraud medium with chloramphenicol. The reverse shows no pigments or exudates. The filaments are septate, branched with a conidiophore bearing a uniseriate aspergillus head. The conidia are globose, smooth, of variable size, and beige-brown. The second isolate shows a crop with medium growth presenting a forest aspect seen from above, powdery and sometimes round in the center but insular at the edges. The color of the culture is dark green, mixed with white in small amounts. The back of the dish does not show pigments and exudates. Microscopic observation showed septate, unbranched filaments, a biseriata aspergillus head with a large vesicle, and globose, smooth conidia of variable sizes.

5.1.2.2. *Aspergillus* Section Nigri

Three species of *Aspergillus* section nigri have been observed in oilseeds and their derived products. The first species presents a concentric culture with four (4) circles of brown, yellow-brown then white color in a centripetal way. This mold has a downy to powdery appearance with branched furrows. The border is perfectly round and the

reverse of the box has presented a yellow-gold pigment. The spore-bearing organ of this mold is a long, smooth, colorless conidiophore. The filaments are very slightly septate and unbranched. The phialides are carried by metulae inserted over the entire upper part of the vesicle. The ex-conidia are small, smooth, and globose. The second species is a colony with the appearance of a young sea urchin. Its culture is slightly domed with a regular contour; it has average growth on Sabouraud chloramphenicol agar. On the reverse, the box shows pale yellow pigmentation. The filaments of the third isolate are long, branched, and septate. The sporiferous organ is a conidiophore bearing a poorly developed spherical vesicle with a biseriata aspergillus head. The conidia are globose and of variable sizes. The fourth species of *Aspergillus* section nigri found in oilseeds and their by-products have a fluffy appearance and a variable color (white, yellow, brown). Its culture is non-invasive, domed, and has a regular border with average growth on Sabouraud chloramphenicol agar. On the reverse, the box showed pale yellow pigmentation. Its filaments are long, septate-branched, and well-developed. The sporiferous organ is a long and wide conidiophore, bearing an aspergillus head, and presenting a small vesicle with phialides and biseriata metule. The conidia are globose, smooth, and shiny.



Figure 4. Microscopic cultural and morphological characteristics of molds genus of *Aspergillus* section nigri (A: macroscopic characteristics; B: microscopic characteristics)

In cereals and by-products, two fungal strains belonging to *Aspergillus* section *nigri* were identified. The first strain exhibited an atypical colony with branched furrows, and arborizing, similar to that of the hairs of an ox. The base of the furrows is dark brown and the culture has general beige color on Sabouraud medium with chloramphenicol. Its growth rate is medium and its relief is flat. The back of the Petri dish showed no pigment or exudate. Microscopic observation showed non-septate, non-branched filaments, and the presence of non-branched conidiophores bearing biserial aspergillus heads at their ends. The conidia are globose, smooth, variable sizes, and military green in color. The second species has a medium growth rate and a round shape, consolidated growth with a sandy appearance. Its relief is flat and its mycelium is beige in color dotted with granules on Sabouraud medium with chloramphenicol. On the back of the Petri dish, there are no pigments or exudates. The sporiferous organ is carried by a conidiophore, similar to a uniseriate aspergillus head comprising a medium-sized vesicle and conidia. This fungus has septate and branched filaments as well as globose, smooth military green conidia of varying sizes dominated by large conidia.

5.1.2.3. *Aspergillus fumigati*

A single species of *Aspergillus fumigati* has been identified only in oilseeds and their derived products. It presented a powdery aspect and a flat relief, a military green color varying to dark green. Its culture is non-invasive, flat, and has a regular border with average

growth on Sabouraud agar. The back of the box was colorless. Its filaments are unchambered, unbranched, and elongated. The sporiferous organ is a smooth conidiophore with an aspergillus head, bearing phialides directly inserted on the vesicle, all organized in a uniseriate structure. The conidia are globose, dense, and grouped in clusters.

5.1.2.4. Molds of the genus *Penicillium*

Two species of *Penicillium* have been identified in oilseeds and their derived products. The first species showed an imperfect outline exhibiting a fluffy, powdery appearance. It was green in color with a white border. Its culture was very small and spread out with average growth on Sabouraud agar. The reverse of the box showed no pigmentation. Under the microscope, its filaments are unseptate and unbranched. The sporiferous organ is a conidiophore bearing phialides inserted against each other, the whole forming an image of a brush or pencil. The conidia are round. Regarding the second species, it presented a fluffy appearance with diffusible pigments. It has a color varying from white when young to green when adult. Its culture is small with a very slow evolution. The back of the box was colorless. The filaments of this mold are unseptate and unbranched. The sporiferous organ is a conidiophore bearing phialides inserted against each other and arranged in whorls at the extremity of the conidiophores; the whole forming a brush image. The conidia are smooth, and small in size, with round and ovoid shapes.

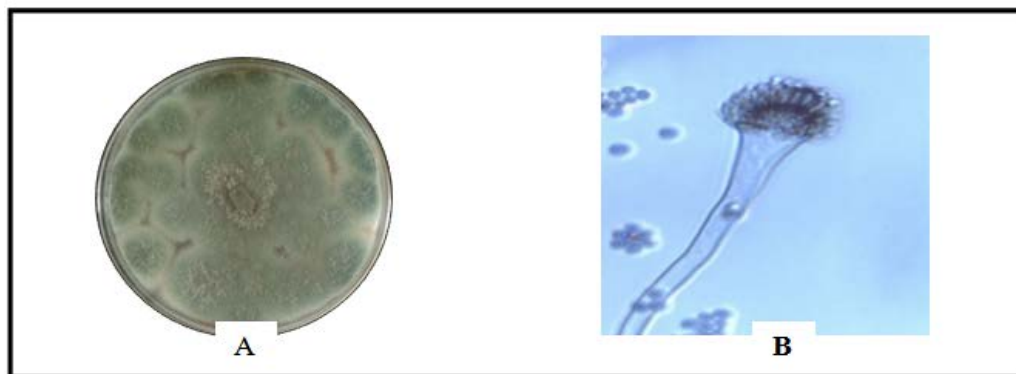


Figure 5. Microscopic cultural and morphological characteristics of molds genus of *Aspergillus fumigati* (A: macroscopic characteristics; B: microscopic characteristics)

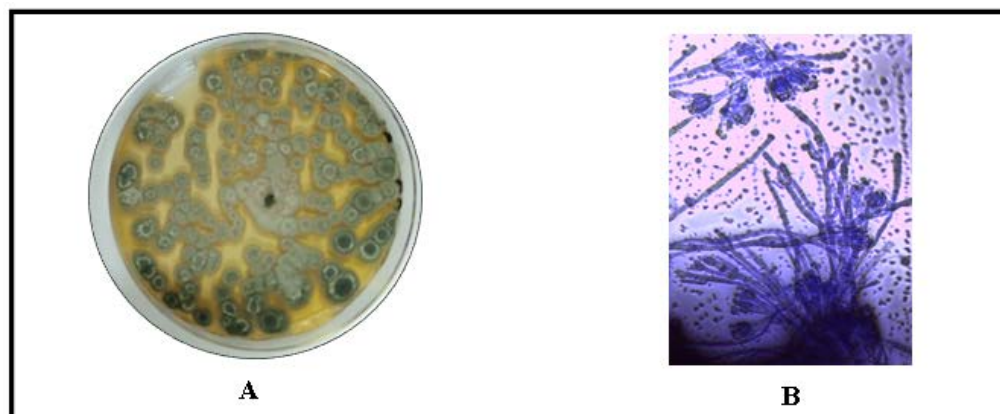


Figure 6. Microscopic cultural and morphological characteristics of molds genus of *Penicillium* (A: macroscopic characteristics; B: microscopic characteristics)

With cereal foodstuffs, the isolates observed presented similar characteristics corresponding to a single species of *Penicillium*. Its culture is roughly circular and insular, beige military green in color with an average growth rate and a flat relief. At the microscopic level, the filaments are branched and septate with sections of reduced sizes. The sporiferous organ presented a head in the form of a bi-verticillate brush. The spores are globose, variable sizes, smooth, and incolor.

5.1.2.5. Molds of the Genus *Fusarium*

Two species of *Fusarium* have been isolated from oilseeds and their derived products. Figure 7 shows the first, which has an average growth rate, a perfectly round, concentric culture with two circles, and a rosaceous large surface. Its appearance is fluffy and looks like the lawn of a football stadium. The reverse of the box has a whitish-pink pigmentation. The filaments of this mold are short, branched, and septate. There are several budding sites for the production of conidia. The ex-conidia are colorless and canoe-shaped in lateral view. The second specie of *Fusarium* was found in oilseeds, and their product is a very small crop with irregular borders and a very rough surface. Its growth rate is very slow. The back of the box shows neither pigmentation nor exudation. The filaments of this isolate are branched and septate. Conidiogenesis takes place in the solitary thallic mode with the formation of aleuria arising directly on the sides of the vegetative filaments or at the end of fine conidiophores. The conidia are ovoid with pointed or rounded ends. Only one specie of *Fusarium* has been identified in cereals and their derived products. This mold has a cottony appearance with medium growth, insular and circular, with non-rectilinear furrows and white then pinkish in color with a flat relief on the Sabouraud medium with chloramphenicol. The back of their boxes did not show any pigment or exudate. Microscopic observation shows septate and branched filaments. The conidia have a fusiform and oval shape attached to the filaments.

5.1.2.6. Molds of the Genus *Rhizomucor*

A single species of *Rhizomucor* has been identified in oilseeds and their by-products on the one hand and cereals

and their derivated products on the other. At the level of oilseeds and their derived products, the species appears on the front of the invasive Petri dish with a high growth rate. Its appearance is cottony, its color is graying and its relief is raised. On the reverse side of the culture, there is no production of pigment or exudate. The filaments are neither septate nor branched. However, we note the presence of rhizoids and stolons. The sporocystophore is in the form of a sporangiophore ending in a globose columella bearing a sporangium at its tip. The sporangium like a spherical bag carries within it endo-conidia called sporangiospores of variable shapes, irregular surfaces, and gray color. *Rhizomucor* observed in cereals and their by-products show a cottony colony resembling very frizzy discolored human hair with very rapid growth, circular with a degraded brown color, and raised relief on Sabouraud chloramphenicol medium. The reverse of the box does not show any pigment or exudate. At the microscopic level, the filaments were non-septate and weakly branched. The sporiferous organ is a sporangiophore. The conidia are globose, smooth, and variable in size.

5.1.2.7. Molds of the Genus *Chrysosporium*

In oilseeds and their derived products, only one specie of *Chrysosporium* has been identified. It presents a perfectly round, non-invasive culture. White in color, it looks like a snowflake. This mold has a slow and not very extensive growth rate. The back of the box is colorless. Its filaments are unseptate and unbranched. From the sporiferous organ arise terminal, lateral aleuria, arranged in a chain and separated by empty segments. The conidia or spores are thick-walled, smooth, pear-shaped aleuria.

Like oilseeds, only one specie of *Chrysosporium* has been observed in cereals and their derived products. Its colony is perfectly circular and concentric (2 circles), Nanking yellow (circle 1), and beige (circle 2). Its appearance is woolly with a flat relief and an average growth rate. The reverse of the box has a yellow pigment. Under the microscope, the filaments are poorly differentiated; the conidia are attached to the filaments. They have a thick wall with a truncated base and are organized in short terminal chains.

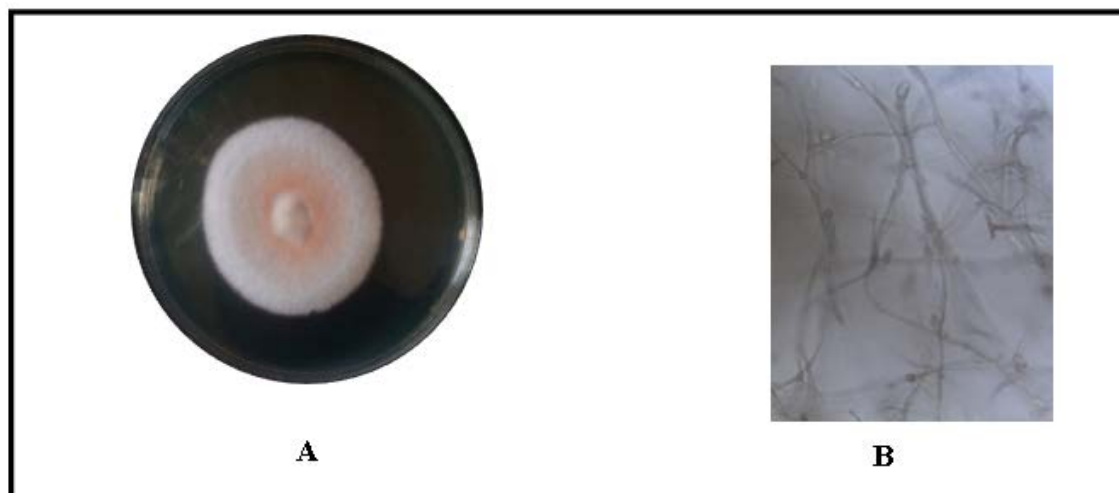


Figure 7. Microscopic cultural and morphological characteristics of molds genus of *Fusarium* (A: macroscopic characteristics; B: microscopic characteristics)



Figure 8. Microscopic cultural and morphological characteristics of molds of genus *Rhizomucor* (A: macroscopic characteristics; B: microscopic characteristics)

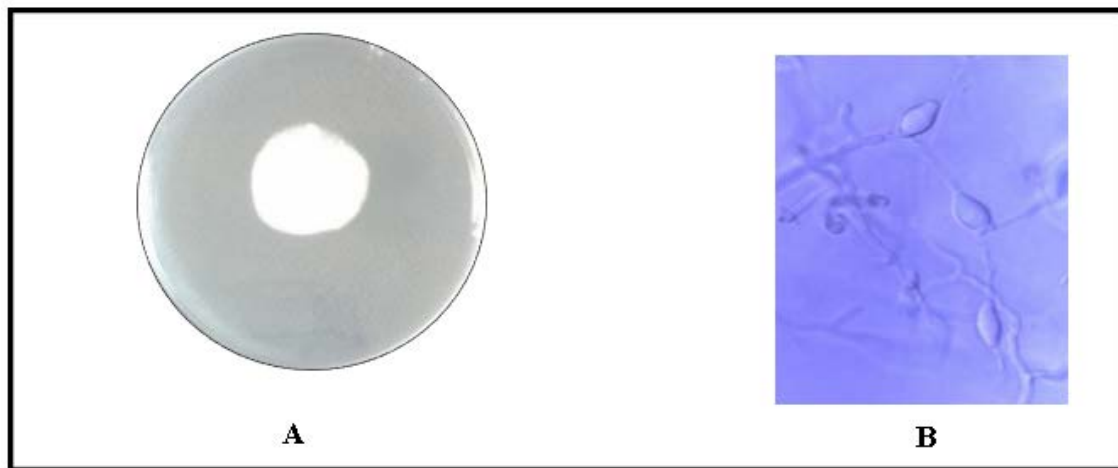


Figure 9. Microscopic cultural and morphological characteristics of molds of *Chrysosporium* genus (A: macroscopic characteristics; B: microscopic characteristics)

5.2. Frequency of Contamination of Groundnut Seeds, Pistachio Seeds, and Their Derivated Products

5.2.1. Frequency of Contamination of Groundnut Seeds and Derived Products by Detected Molds

The molds isolated from groundnut seeds and derived products collected on the markets of Abobo and Adjamé are presented in Table 1. The table shows a diversity of fungal isolates contaminating these products. A total of 14 morphologically different mold isolates were detected. The frequency of detection of these isolates varied from 17 to 100 %. Two isolates of *Aspergillus* are almost always present with a contamination frequency of 100%. Two other isolates of *Aspergillus* and only *Fusarium* were rarely detected in a single product out of the 6 analyzed regardless of the collection municipality.

5.2.2. Frequency of Contamination of Pistachio Seeds and Pastes by the Molds Detected

Table 2 presents the results of mycological analyzes of pistachio seeds and paste collected in the municipalities of Abobo and Adjamé. A total of 13 morphologically

different fungal isolates were detected in pistachio seeds and derived paste. The two isolates *Mucor* and *Rhizopus* were the most present in all pistachio products with a frequency of 100 % and 4 fungal isolates were detected in one product regardless of the collection commune.

5.2.3. Level of Fungal Contamination of Oilseeds and Derived Products According to the Town

Table 3 presents the levels of contamination of oilseeds and derived products analyzed according to the collection communes.

Regarding groundnut-based products, 8 isolates were detected on the seeds collected in the commune of Adjamé with a level of contamination of 38.09 %, while those collected in Abobo were contaminated by 5 isolates with a contamination level of 23.80%. The groundnut pastes collected in the markets of Abobo were contaminated by 15 isolates, i.e. a level of contamination of 71.43 %, while those collected in Adjamé presented a level of contamination of 61.90 %. Finally, the *lidèguè* product collected in Adjamé recorded a contamination level of 52.38 % with 11 fungal isolates detected, while only 5 fungal isolates were detected in the *lidèguè* collected in Abobo, i.e. a contamination level of 23.80 %.

Table 1. The frequency of contamination of groundnut seeds and derived products collected on the markets of the communes of Abobo and Adjame by an isolate detected

Isolate of mold	Communes						Number of contaminated products	Frequency of contamination (%)
	Abobo			Adjame				
	Peanuts and derived products							
	Seeds	Pasta	Lidêguê	Seeds	Pasta	Lidêguê		
<i>Aspergillus</i>	-	-	-	-	-	+	1	16,67
<i>Aspergillus</i>	-	-	-	-	-	+	1	16,67
<i>Fusarium</i>	-	-	+	-	-	-	1	16,67
<i>Aspergillus</i>	-	+	-	-	-	+	2	33,33
<i>Rhizomucor</i>	-	+	-	-	+	-	2	33,33
<i>Chrysosporium</i>	-	+	-	-	-	+	2	33,33
<i>Penicillium</i>	-	+	-	-	+	+	3	50,00
<i>Aspergillus</i>	-	+	-	+	-	+	3	50,00
<i>Mucor</i>	-	+	+	+	+	-	4	66,67
<i>Mucor</i>	+	+	-	+	+	-	4	66,67
<i>Rhizopus</i>	+	-	-	+	+	+	4	66,67
<i>Penicillium</i>	-	+	+	+	+	-	4	66,67
<i>Aspergillus</i>	+	+	+	+	+	+	6	100,00
<i>Aspergillus</i>	+	+	+	+	+	+	6	100,00

Total number of isolates = 14

(+) presence of the isolate; (-) absence of the isolated.

Table 2. The frequency of contamination of pistachio seeds and paste collected on the markets of two municipalities of Abidjan by each isolate of mold detected

Molds isolates	Communes				Number of contaminated products	Isolation frequency
	Abobo		Adjame			
	Pistachio and derived products					
	Seeds	Pasta	Seeds	Pasta		
<i>Fusarium</i>	-	-	-	+		
<i>Aspergillus</i>	-	-	-	+	1	25
<i>Aspergillus</i>	-	-	-	+	1	25
<i>Aspergillus</i>	+	-	-	-	1	25
<i>Aspergillus</i>	-	+	-	+	2	50
<i>Chrysosporium</i>	+	+	-	-	2	50
<i>Penicillium</i>	-	+	-	+	2	50
<i>Aspergillus</i>	+	+	-	-	2	50
<i>Aspergillus</i>	-	-	+	+	2	50
<i>Aspergillus</i>	+	+	-	+	3	75
<i>Penicillium</i>	+	+	-	+	3	75
<i>Mucor</i>	+	+	+	+	4	100
<i>Rhizopus</i>	+	+	+	+	4	100

(+) presence of the isolate ; (-) absence of the isolate.

Table 3. Distribution of contamination levels of oilseeds and derived products collected by molds according to the communes of Abobo and Adjame

Oilseeds	Groundnut						Pistachio			
	Seeds		Pastes		Lidêguê		Seeds		Pastes	
Communes of sampling	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad
Number of isolates	5	8	15	13	5	11	10	3	10	15
Fungal contamination level (%)	23,8	38,09	71,43	61,9	23,8	52,38	55,55	16,66	55,55	83,33
Total contamination level (%)	38,09		85,71		66,66		44,44		94,44	
Oilseeds	Groundnut						Pistachio			
Derivated products	Seeds		Pastes		Lidêguê		Seeds		Pastes	
Communes of sampling	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad
Number of isolates	5	8	15	13	5	11	10	3	10	15
Fungal contamination level (%)	23,8	38,09	71,43	61,9	23,8	52,38	55,55	16,66	55,55	83,33
Total contamination level (%)	38,09		85,71		66,66		44,44		94,44	

(Ab): Abobo; (Ad): Adjame.

At the pistachio level, the seeds collected in Abobo were contaminated by 10 isolates, i.e. a level of contamination of 55.55 %, while only 3 fungal isolates (16.66%) were detected in those collected in Adjamé. Fifteen isolates of molds were found in the pistachio paste of Adjamé (83.33) against 10 in that of Abobo with a level of contamination of 55.55%.

The table also presents the level of overall fungal contamination of oilseeds and derived products collected jointly in the two communes. The results obtained indicated that at the groundnut level, the paste, the lidêguê product, and the seeds were contaminated by 18 isolates (85.71 %), 14 isolates (66.66%), and 8 isolates (38.09%) respectively.

Regarding the pistachio, the pips were contaminated by 8 fungal isolates, i.e. 44.44 %, while 17 fungal isolates were found in the dough, i.e. a level of contamination of 94.44 %.

5.2.4. Frequency of Distribution of Molds Isolated from Maize and Millet Seeds

The microbiological analysis carried out on maize and millet grains revealed strong fungal contamination

(Table 4). Of a total of 36 fungal strains isolated, 61.11% contaminated the maize grains collected in the commune of Abobo. On the other hand, millet grains collected in this commune, maize, and millet grains sampled in the commune of Adjamé were contaminated, for each type of grain, by 55.56 % of different fungal isolates. Based on the total samples, it appears a predominance of molds of the genus *Aspergillus*, followed by the genera *Rhizopus*, *Mucor*, *Rhizomucor*, *Chrysosporium*, *Fusarium*, and *Penicillium*.

5.2.5. Frequency of distribution of molds isolated from maize and millet flour

Table 5 presents the microbiological analysis of the flours sampled in the communes of Abobo and Adjamé. This analysis revealed that the commune of Abobo recorded the highest percentage of contamination at the level of these flours, including 50% of fungal isolates for corn flour and 44.44% for millet flour. On the other hand, the commune of Adjamé recorded a lower percentage with 36.11% for corn flour and 38.89% for millet flour. Of the 48 samples analyzed, molds of the *Aspergillus* genus were predominant while these flours were less contaminated by molds of the *Penicillium* genus.

Table 4. Frequency of distribution of molds isolated from maize and millet seeds collected in the markets of two municipalities of Abidjan (Abobo and Adjamé)

Mold isolate	Communes of sampling			
	Abobo		Adjamé	
	Maize seeds	Millet seeds	Maize seeds	Millet seeds
<i>Aspergillus</i>	17	14	16	15
<i>Penicillium</i>	0	2	0	1
<i>Fusarium</i>	1	0	0	0
<i>Rhizopus</i>	1	1	1	1
<i>Mucor</i>	1	1	1	1
<i>Rhizomucor</i>	1	1	1	1
<i>Chrysosporium</i>	1	1	1	1
Number of molds isolated	22	20	20	20
Percentages of fongic isolates (%)	61,11	55,56	55,56	55,56

Table 5. Frequency of distribution of molds isolated from maize and millet flour collected in the markets of two municipalities of Abidjan (Abobo and Adjamé)

Mold isolated	Municipality of sampling			
	Abobo		Adjamé	
	Maize flour	Millet flour	Maize flour	Millet flour
<i>Aspergillus</i>	13	9	9	9
<i>Penicillium</i>	0	2	0	1
<i>Fusarium</i>	0	1	0	0
<i>Rhizopus</i>	1	1	1	1
<i>Mucor</i>	1	1	1	1
<i>Rhizomucor</i>	1	1	1	1
<i>Beauveria</i>	1	0	0	0
<i>Chrysosporium</i>	1	1	1	1
Number of isolates	18	16	13	14
Percentages of molds isolated	50,00	44,44	36,11	38,89

5. Discussion

The phenotypic analysis based on the microscopic cultural and morphological characteristics of the molds made it possible to highlight 14 isolates of molds in oilseeds and their derived products. On the other hand, 36 fungal isolates have been identified in cereals and their derived products. All these isolates were grouped into 7 mold genera, namely *Mucor*, *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizomucor*, *Rhizopus*, and *Chrysosporium*. Previous studies identified the genera *Mucor*, *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus* but not the genera *Rhizomucor* and *Chrysosporium* [9,20,21,22,23]. The wide fungal diversity observed in both oilseeds and cereals and their derived products could be related to cultural practices. Indeed, some studies have shown that the soil is a key influence on fungal contamination in agricultural produce [1,24]. A study conducted by [25] revealed that monocultures or combination cropping can promote mold growth in oilseeds. In addition, several studies have shown that practices during harvest and after harvest can favor the development of molds in oilseeds, cereals, and their by-products [22,26,27]. It was demonstrated that traditional processing plants favor fungal contamination and are more conducted to the contamination of cereals, oilseeds, and their products compared to mechanized processing plants [2]. Furthermore, during storage, storage techniques and relative humidity during storage can accentuate the contamination of cereals, oilseeds, and their by-products [1,22,28].

[29] argue that the contamination of seedlings by insects and rodents is a gateway to fungal contamination. Molds belonging to the genera *Mucor*, *Penicillium*, *Fusarium*, *Rhizomucor*, *Rhizopus*, and *Chrysosporium*, which are qualified as storage molds, could contaminate oilseeds during storage [2,25].

The wide diversity of mold-contaminating oilseeds and derived products from cross-contamination throughout the value chain at the producer level can reduce both the yield and quality of both products [29]. The results showed the predominance of the strains belonging to the genus *Aspergillus*. These results are consistent with those obtained previously [2,9,23,30]. According to previous studies, heavy contamination by several species belonging to the genus *Aspergillus* can lead to aflatoxin contamination of crops and lead to significant annual financial losses. Indeed, groundnut pods and seeds can be contaminated with aflatoxins in the soil before, during, after harvest, and during storage [31,32,33]. As far as products derived from oilseeds are concerned, they are also highly contaminated by numerous fungal genera. This high fungal contamination could be to the artisanal techniques of processing the seeds into oilseed pastes [25]. However, it should be noted that groundnut seeds and derived products were more contaminated by molds than pistachio-based products. This difference in the level of contamination could be explained by the biological organization of the products [7,9]. Indeed, groundnut seeds are contaminated by molds from sowing to processing [25] that are enclosed in a melon or cucumber. The detection of molds in *lidèguè* could be explained on

the one hand by contamination during storage and on the other hand by ingredients such as corn flour added during the manufacture of the product [1,24].

Mucor and *Rhizopus* genera were found more in all analyzed pistachio products with 100% frequency. The presence of these molds in “African pistachio” products would be linked to the environment in which these products are kept and sold. Because previous studies have shown that molds of the order Mucorales are permanently in the human environment [34,35,36]. These works revealed that the molds of the order Mucorales are generally considered spoilage agents. Those mold genera do not produce mycotoxins [36]. The pastes of the two oilseeds are more contaminated by molds, more specifically by molds belonging to the *Chrysosporium* genera. This presence can be explained by a lack of hygiene in the production units. Indeed, the genus *Chrysosporium* is a keratinophilic filamentous fungus commonly isolated from the soil where it lives on the remains of hairs and feathers. *Chrysosporium* species are occasionally isolated from nail scrapings and skin, especially from feet, and may cause skin infections and onychomycosis in animals and humans [37]. The level of contamination of oilseeds and derived products varied according to the collection municipalities. Among the two (2) collection communes, Adjamé presented a high level of contamination of groundnut seeds as well as *lidèguè* compared to the commune of Abobo. On the other hand, the pulp collected in Abobo was more contaminated than that collected in Adjamé. These differences may be due to environmental (places and storage conditions) and human factors. Indeed, the fungal diversity contaminating derived products and dry seeds is specific to each municipality. The development of microorganisms can be influenced by the geographical origin of the product as reported by [38]. The contamination observed in cereals and their by-products would also be linked to post-harvest treatments as indicated above.

Among the molds contaminating maize and millet grains and flour, are those of the genera *Aspergillus*, *Penicillium*, and *Fusarium*. Some species of those genera are potential producers of mycotoxins [33,39,40,41]. The predominance of molds belonging to the genus *Aspergillus* (section *flavi* and *nigri*) with more than 72% of the mycoflora of grains and products derived from maize and millet requires special attention and monitoring since they are also known to produce aflatoxins [42]. According to [43], some species of *Penicillium* are known for their potential to produce toxins such as ochratoxins and citrinin. As for molds of the genus *Fusarium*, they have very often been reported as flora present in cereal grains. In addition, they are known for their ability to produce a variety of mycotoxins including fumonisins, moniliformin, fusaric acid, beauvericin, and deoxynivalenol [2,41,44].

Knowing the many populations of all ages in the two target towns of this study, it is to be feared that food poisoning occurs frequently due to the ingestion of these two kinds of cereal and derived products. The infant population whose complementary foods are mainly porridge made from millet and/or maize flour is more exposed to the dangers of mycotoxins contamination and other diseases.

6. Conclusion

The objective of this study was to contribute to the identification of the genus of molds contaminating groundnut seeds, pistachio seeds, maize, millet, and grains in bulk, and their derived products commonly consumed in Abidjan, Côte d'Ivoire. At the end of this work, the results obtained indicated that twenty-seven (27) mold isolates were detected in the various products collected in the two communes of Abidjan. These mold isolates showed different cultural and microscopic morphological characteristics that allowed the identification of spoilage mold genera such as *Mucor*, *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizomucor*, *Rhizopus*, and *Chrysosporium*. Molds of the *Aspergillus*, *Penicillium*, and *Fusarium* genera are known to produce mycotoxins. Mold strains belonging to the genus *Aspergillus* were dominant. The common storage and production of oilseeds-derived products influenced the fungal community composition of the products. Thus, the oilseeds collected in the commune of Adjamé were more contaminated by the majority than those collected in the commune of Abobo.

Conflict of Interests

The authors have not declared any conflict of interest.

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