ISOLATION AND PHENOTYPIC IDENTIFICATION OF SOME MOLDS ISOLATED FROM PEANUT PASTES SOLD IN SOME PUBLIC MARKETS IN THE CITY OF DALOA (CENTRAL-WEST, CÔTE D'IVOIRE)

ABSTRACT

Molds are common contaminants in peanut pastes.Their presence can have a more or less serious effect on the health of the consumer.The general objective of this work is to evaluate the diversity of filamentous fungi producing mycotoxins in peanut paste sold in public markets in the city of Daloa.A total of fifty samples were collected from five markets for carrying out the work.Isolation was carried out on Sabouraud medium with chloramphenicol.An identification of filamentous fungi was carried out through the analysis of macroscopic and microscopic morphological characters.Of the 50 samples, ten do not contain mold with a rate of 20% and 40 contain mold with a rate of 80%.Macroscopic analysis after enumeration made it possible to identify two species of mold.These are *Aspergillus niger* and *Aspergillus flavus*.Microscopic examination made it possible to partially confirm its two species.The presence of these species with their mycotoxin production capacities suggests that contaminated peanut paste represents a risk to the health of consumers.

Keywords: Aspergillus, consumers, mycotoxins, peanut paste

1. INTRODUCTION

Peanut (*Arrachis hypogaea*) is an annual legume whose fruit ripens in the ground [1]Its seeds are mainly used as raw material for the extraction of oils used as seasoning and in cooking and to produce peanut butter.It can be eaten as roasted peanuts or used as a condiment, particularly in the preparation of famous sauces [1].Peanut, the twelfth largest crop production in the world, is a major crop in most tropical and subtropical regions.It is cultivated on all continents, in approximately 120 countries, over a total area of 24.6 million hectares for a production of 38.2 million tonnes[1].The largest peanut producing countries are found on the Asian, African and American continents [2].The Asian continent, with 13.3 million hectares, accounts for half of the world's areas planted with peanuts.China with 13 million tonnes) and India (9 million tonnes) are the world's leading producers with production which contributed more than half of world production in 2007. The USA with 2.3 million tonnesare the leading producing country on the American continent.The African continent, with its 10 million

hectares of surface area occupied by peanut cultivation and its 10 million tonnes, occupies second place ahead of the American continent.Peanut production on the African continent has experienced significant growth since the early 1990s [3].This growth is mainly linked to the increase in production in West African countries [4]. With an estimated production of 150,000 tonnes, Côte d'Ivoire is the 17th largest producer of peanuts in the world [5].Peanut sauce, along with seed sauce and eggplant sauce, constitutes one of the three sauces that are served in almost all restaurants in the towns of Côte d'Ivoire [5,6] .The center and the north constitute the main production areas in Côte d'Ivoire [5].Despite its multiple nutritional and economic advantages, peanut paste is susceptible to attack by several microorganisms which are present in natural ecosystems such as air, soil and water.They are also present on man himself and on all living animal and plant beings.As a result, all food products, whether processed or not, can be contaminated by microorganisms [7].Contamination of foodstuffs can have a more or less serious effect on the quality of the product and the health of the consumer.It can be the cause of a deterioration of the product, causing it to lose its organoleptic and/or commercial characteristics and sometimes the cause of serious poisoning or toxic infections.These microorganisms proliferate in the body, multiply and produce disorders.It is a condition, generally infectious or toxic in nature [8]. Peanut seeds are most often subject to microbiological contamination, particularly fungal contamination.More than 300 mycotoxins are currently identified internationally, they are produced by some 200 varieties of toxic fungi [9].Aflatoxin is a highly carcinogenic toxin transmitted by saprophytic soil fungi of the genus Aspergillus such as A. flavus and A. parasiticus[2].Contamination of peanuts by aflatoxin is today a major public health problem.In fact, more than twenty aflatoxin molecules are known today with a wide distribution and a high contaminating power.The best known are BI, B2, G1 and G2 [10]. In Côte d'Ivoire and particularly in Daloa, there is no data on contamination by toxin-producing molds in peanut pastes sold on public markets and particularly those relating to the presence of toxin-producing molds.aflatoxins.There is also no monitoring providing statistics on illnesses resulting from the consumption of peanut grains in general and peanut pastes in particular contaminated by these microorganisms.It is also with the aim of overcoming the problems caused by these microorganisms that this scientific investigation was initiated.The general objective of this study is to evaluate the diversity of filamentous fungi in peanut pastes sold in the different public markets of Daloa.

2. MATERIALS AND METHODS

2.1.Sampling

Peanut pastes sold in public markets in the city of Daloa were used to carry out this study.A total of fifty (50) samples of peanut pastes collected by purchase on the markets were the subject of this study.These are the Abattoir Market, Grand Marché, Tazibouo Market, Lobia Market, Kennedy Market, Sun Market and Orly Market.The collected samples were transported in a cooler containing ice accumulators to the laboratory for analysis.

2.2.Microbiological analysis

2.2.1.Preparation of culture media

The different media used to carry out the work were prepared according to the manufacturer's recommendations mentioned on the different boxes.These are the Eau Tamponnée broth used in the enrichment phases and the Sabouraud Chloramphenicol agar [11]used for the isolation of molds.

2.2.2.Preparation of the inoculum

Ten (10 g) grams of the peanut paste are weighed aseptically using a balance in an Erlenmeyer flask.A volume of 90 ml of buffered peptone water is added.The mixture was homogenized for 2 min to obtain the mother suspension which is the inoculum.This suspension is left on the bench for 30 min at laboratory temperature, to allow the microorganisms to multiply.

2.2.3.Seeding and incubation

After revivification, and using a platinum loop, seeding took place.To do this, the platinum loop is introduced into the inoculum then seeded in tight streaks on the Sabouraud Chloramphenicol medium prepared and poured in advance at a rate of 20 mL per box.The boxes thus inoculated are incubated at 26 °C ± 1 for 3 to 5 days.

2.2.4.Isolation of filamentous fungi

Isolation was carried out on Sabouraud medium with chloramphenicol according to the work of [12,13].Colonies selected on the different Petri dishes were subcultured on Sabouraud chloramphenicol medium and incubated at 37°C for 24 hours.These young colonies were used for identification.

2.2.5.Purification and conservation of strains

The strains identified as presumed filamentous fungi were purified again on Sabouraud medium with Chloramphenicol.Purification consisted of streaking each strain identified on the Petri dishes to ensure that there was no contamination.After 24 hours of incubation at 37°C, the strains obtained are pure.They are preserved in test tubes containing sterile distilled water for further work using the technique of [13].Thus, 25 isolates of filamentous molds belonging to the genus Aspergillus sp were kept for further work.

2.2.6.Analysis of the macroscopic characteristics of the isolates

The isolates obtained were subcultured again on Sabouraud Chloramphenicol agar.Identification is done by eye and is essentially based on cultural characteristics.After culturing the isolates for 24 hours at 37°C on Sabouraud Chloramphenicol medium, several aspects of the vegetative system are observed [14].

2.2.7.Analysis of microscopic characteristics of isolates

Microscopic identification is carried out by taking a small mycelial fragment using a small piece of adhesive tape from young colonies after 24 hours of incubation at 37°C.This tape is applied by the sticky side to the colony then placed on a slide.Then the fragment is placed on a slide by adding ammoniacal Congo red and is then covered with a coverslip.Finally, the observation is carried out using an optical microscope at different magnifications (G×10, G×40) as well as immersion (G×100) [14].

3. RESULTS

3.1.Diversity of filamentous fungi isolated from peanut pastes analyzed

3.1.1.Macroscopic observation of cultured colonies

The macroscopic analysis carried out on the colonies obtained after isolation on SabouraudChloramphenicol agar revealed that the majority of the peanut paste samples analyzed are contaminated by molds.Furthermore, a gender was formally observed.This is the genus Aspergillus sp, the colonies observed are in a downy form.In addition, the thallus is hyaline and presents a septate mycelium ending in numerous well-erect conidiospores.It should be noted that its conidiospores are terminated by vesicles. Furthermore, careful observation of the colonies made it possible to distinguish two species of Aspergillus.The colonies observed have whitish colors then yellow on the reverse.Growth is very rapid and on the reverse side the colony is colorless to pale yellow, it is Aspergillus sp 1 (Figure

1A).Furthermore with the color of the whitish colonies, a rounded and cottony appearance, then yellow on the reverse.The reverse side with rapid and invasive growth has yellow pigment and a diameter greater than or equal to 3 mm.This is Aspergillus sp 2 (Figure 1 B).

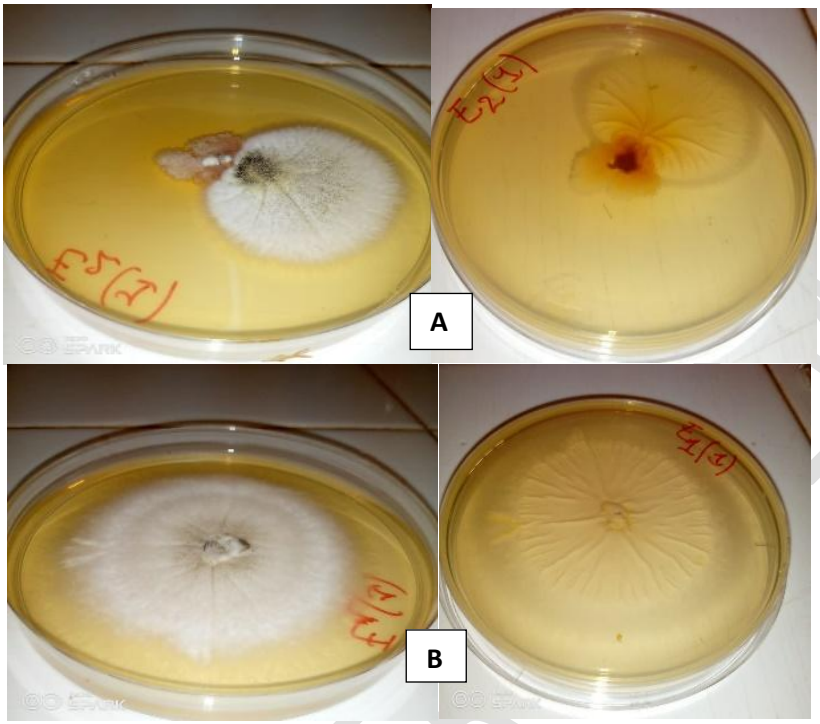

Figure 1: Mold colonies observed on Petri dish A: Aspergillus sp 1, B: Aspergillus sp 2

3.1.2.Microscopic observation of cultured colonies

Methylene blue staining made it possible to identify the isolates from the peanut paste samples.Furthermore, the microscopic examination made it possible to confirm the genus *Aspergillus* identified during the macroscopic examination.This was the genus *Aspergillus sp* 1 (Figure 2 A) which has Thalles with septate mycelium carrying numerous unbranched conidiospores, ending in vesicles.They have phialides formed directly on the vesicle with biseriate conidial heads.The conidial mass is radiating and the conidia are formed in single-celled chains.It is of the genus *Aspergillus sp 2* with aspergillary, uniseriate and radiate heads and long, hyaline and non-septate conidiospores.The conidia are globose and pale green in color (Figure 2 B).

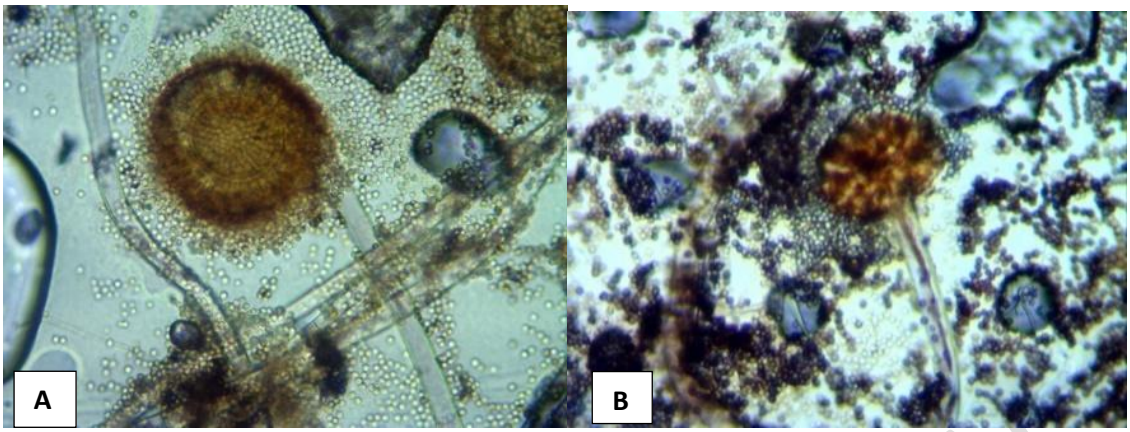

Figure 2: Aspergillus sp1 (A) and Aspergillus sp2 (B) observed under an optical microscope

3.2.Prevalence of *Aspergillus sp* species identified in peanut paste samples

Taking into account microscopic and macroscopic analyses, two species of aspergillus were identified out of the 25 isolates extracted.This is Aspergillus niger with 15 isolates which was identified with a rate of 60%.It should also be noted the identification of 10 species of Aspergillus flavus with a rate of 40% (Table 1).

Table 1: Identification of the species of the genus Aspergillus and frequency of isolation

Genus Aspergillus sp	**Macroscopic appearance**	**Microscopic appearance**				**Species name**	**Isolation frequency %**
		Aspergillus head	**Conidiophore**	**Vesicle**	**Conidia**		
*Aspergillus*sp 1	**Reverse:** White then green, green-gray then dark green to blackish-gray **Verso:** colorless, yellow, green or red-brow	Disbarred Biseriate	Long: 1.5-3mm Wide 15-20µm Smooth Colorless to yellow brown	Spherical (25-90µm)	Large globose conidia (3.5-5µm), brown,	*Aspergillus niger*	15 (60 %)
*Aspergillus*sp 2	**Reverse:** Fluffy to powdery, white then yellow to yellow-green **Verso:** colorless, pinkish to dark red-brown	Disbarred Plain or Biseriate	Long (up to 2.5mm), Often warty colorless Thick walls	Spherical (23-42µm)	Large globose conidia (3-4µm) to subglobose, pale green,	*Aspergillus flavus*	10 (40 %)

4. DISCUSSION

In this study, there was the presence of molds and yeasts in the peanut paste with average loads higher than the microbiological criteria set at 103 CFU/g.Generally speaking, all of these contaminations would reflect a lack of hygiene in food manufacturing [15].Regardless of the origins of the peanut pastes, the average contaminant loads were well above the prescribed standards.The massive presence of these potentially pathogenic germs in the various peanut pastes constitutes a danger for human health because they can lead to the production of toxins.The search for germs made it possible to highlight microorganisms of the fungal flora.This presence may be due to contamination of the samples by soil during drying and storage, by air, insects, water.In poorly hydrated foods such as cereals or coffee beans, bacterial growth is always very slow and it is molds that become the main players [16].This flora could undoubtedly represent the microorganisms that the food industry faces.Furthermore, foods are rarely sterile.They usually contain microorganisms which are mostly harmless;some of them are useful.This is the case for many charcuterie, dairy or vegetable products (bread, sauerkraut, beer, wine) for which the microbial flora is said to be positive [17].Furthermore, microscopic fungi can have a negative effect on food.These are spoilage microorganisms which can cause organoleptic or nutritional damage (fermentation or development of undesirable aromas) and lead to a reduction in the shelf life of foods [18]. The analysis of peanut pastes sold in the public markets of Daloa revealed the presence of many different fungal species.The samples analyzed were found to be contaminated by fungi with a prevalence of 80% for the genus Aspergillus, spp and other unidentified germs.These results are comparable to the work of [19] who specifies that these are mainly storage molds such as *Aspergillus*, *Penicillium* and *Mucor* and *Eurotium* (68%).These fungal genera are present in the majority of soils of all types.[20] stated that *Aspergillus*, *Penicillium, Fusarium, Trichoderma, Mucor, Absidia, Rhizopus* are autochthonous strains, usually isolated from most terrains.It was even reported that Aspergillus and Penicillium were the species mainly isolated from the waters of Lake Oubeira (PNEK) with a percentage of 50% and 19%, respectively by [21].The results obtained in this study are therefore in agreement with those of [22], who was able to isolate the genus *Aspergillus* mainly with a frequency of 37.5% bringing together two species including: *Aspergillus flavus* and *Aspergillus niger*, from arid soil and with a frequency of 42.85% from aother CHEGGA soil.A*spergillus* have a wide geographic distribution, but are more often associated with regions with warm climates [23].They grow on decomposing organic matter, in soil, compost, foodstuffs, cereals.Many species of

Aspergillus are present in the human environment, particularly in dust and air [24,25].Certain species can be directly pathogenic for humans and animals by being capable of invading living tissues and causing aspergillosis, in this case, *Aspergillus fumigatus* responsible for pulmonary mycoses and Aspergillus niger responsible for aspergillosis of the ear canal [24,26]. Many species of *Aspergillus* are also known for their ability to produce mycotoxins responsible for animal and human pathologies. In addition, certain species of Aspergillus are used in the food industry andin the biotechnological products industry, particularly for the production of enzymes and organic acids [27,7]. The Genus Penicillium brings together filamentous fungi, belonging to the phylum Ascomycetes. Thisgenus includes approximately 227 species defined essentially according to the characteristics of the thallus, penicils and spores [28]. *Penicillium* are fungi, most of them very common in the environment, polyphagous, which can be responsible fornumerous damages.Their natural habitat is soil, foodstuffs, organic matter.

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