

Original Research Article

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Diversity of Mold Associated with the Rotting of Peanut (*Arachis Hypogaea*) during Storage in Some Markets of Douala Cameroon

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ABSTRACT

Keywords

Pathogens, *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor*, *Rhizopus stonifer* and *Rhizoctonia solani*

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During storage, peanut seeds face many problems including deterioration that creates favorable conditions for mold growth and mycotoxin production. This study describes the diversity of molds responsible for groundnut rot during commercialized in the city markets of Douala-Cameroon. Peanut samples were collected in a ten markets in the city of Douala. It has been noted that the postharvest fungal flora of groundnuts in the city of Douala is composed of various genre of molds. Many fungal species have been isolated and identified on peanut seeds commercialized in the study markets of the city of Douala. A total of six thousand fungal isolates (6000) were obtained in post-harvest culture. The main species are: *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysparum*, *Fusarium solani*, *Fusarium vinale*, *Penicillium*, *Mucor*, *Rhizopus stonifer* and *Rhizoctonia solani*. *Aspergillus* is the most common genus (52%), of all samples whatever the geographic origin. All fungal groups are present in all markets of the city of Douala, and only differ in proportion.

Introduction

In Cameroon, agriculture contributes for about 22.9% of gross domestic product and represents the first employer with 62% of the active population Anonymous (2015). It is based on the growing cereals, starchy plants and legumes. As most of seed legumes, groundnuts occupy a place of choice in human nutrition Kouadio (2007). These seeds contain about 45-50% lipids, 25-30% protein, 12% carbohydrates, 3% fiber, water, minerals and vitamins Annerose (1990).

In Cameroon, groundnuts rank second after cotton in terms of economic profitability and this crop is used both as a cash crop and as a food or subsistence crop Magrin (2003).

Despite its nutritional, economic and positive effects, the different peanut varieties are the preferred substrate for molds, especially the genus *Aspergillus* Schmale *et al.*, (2005). These attacks do not only affect the yield and nutritional value during storage, but also human health. Research studies in West Africa has referred to the attack and loss of stocks of cereals and legumes by insects and

mycotoxins, which in the tropics are responsible for losses of up to 30% Philogène *et al.*, (1989), Ratnadass *et al.*, (1989), Ashamo (2006) Tagne *et al.*, (2003). However, in Cameroon, no work has been done to our knowledge on the diversity of molds that attack peanuts sold in the markets of the city of Douala.

Therefore, the objective of this study was to describe the diversity of molds responsible for groundnut rot during commercialized storage in the city markets of Douala-Cameroon. More specifically, to make a survey of varieties and mode of peanuts contamination in the study markets of Douala; to characterize (morphological and microscopic) the molds responsible for the rot of the different varieties of peanuts (*Arachis hypogaea*) sold in the city of Douala.

Materials and Methods

A survey form was established to collect informations on the sold varieties groundnuts, the conditions and their mode of storage. From the infected peanut seeds used in this study, mold that attack these foodstuffs were isolated and identified.

Peanut sampling

We sampled in ten major markets (Central, Dakar, Deido, Mabanda, Ndogpassi, New – bell, Sandaga, Bonamoussadi, Bonassama, PK 14) of the city of Douala. The collections were made during the period from January to June 2016 for a sample of six thousand (6000) on 5 peanut varieties the most commercialized as show in Figure 1.

Isolation and identification of molds

Isolation of molds was done by direct contact on culture medium using Sabouraud Dextrose Agar (SDA) supplemented with

chloramphenicol as antibacterial according to the method described by Foko and Sougnabe (1991). Infected peanut seeds were seeded aseptically in Petri dishes containing 10 mL of Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol. The culture was incubated during 3-5 days with cooler set at 25 ° C in order to observe the growth of the colonies of which numerous colonies of molds of various genus were observed and the percentage of contamination evaluated.

The operation was repeated many times to obtain pure culture of the isolate. Identity of the mold isolates was based on macroscopic and microscopic criteria as described by Jinyoung Lim *et al.*, (2002), Agrios (2004) and Sameza *et al.*, (2014).

Results and Discussion

The survey of varieties and peanut contamination in the study markets of Douala city revealed that five(5) varieties of peanut exist, three (3) are abundant in the city with a frequency of 47%. The most widely consumed and commercialized varieties are Garoua (40%), Makmak (37%) and Yaounde (20%) (Figure 2A). The most common pathogens that attack groundnut are molds (52%), weevils (19%) and bruchids (17%) (Figure 2B).

Morphological and microscopic characteristics of the main fungal species isolated from peanuts sold in the city of Douala

Many fungal species were isolated and identified on commercialized peanut seeds in the study markets of the city of Douala. The main species are: *Aspergillus flavus*, *Aspergillus niger*, *Fusarium Spp*, *Penicillium sp* and *Rhizoctonia Sp*.

Aspergillus flavus

Aspergillus flavus pure culture has shown that the granular colony is white the first two days and then takes on a yellow, yellow or brown green color; the reverse of the dish is colorless (Figure 3A). Microscopic examination of the culture showed that conidiophores are swollen at the top and have many conidial-bearing cells. Conidia are hyaline and arranged in long chains or individualized (Figure 4A).

Aspergillus niger

Macroscopic examination of *A. niger* shows between the 2nd day a first white thallus then takes towards the 4th day of culture a black shade surrounded with white (Figure 3B). The microscopic observation shows a fan structure with small, round and black spores (Figure 4B).

Fusarium oxysporum

In 4 day culture, the mycelium is cottony and white (Figure 3C). On microscope, two types of conidia are observed: microconidia, short and generally abundant, unicellular and macroconidia must slightly curved (Figure 4C).

Rhizoctonia solani

Rhizotonia solani colonies are cottony with a greyish to black coloring and the coating of the dish dark black (Figure 3D). On microscope, the mycelium is partitioned and presents a slight constriction at the level of the septa; it's provided by with side forming a right angle (Figure 4D).

Penicillium sp.

Penicillium sp. isolate obtained in young culture have a compact thallus, green

surrounded by a white part but that become dark and powdery while getting old (Figure 3E). At the microscopic level, the conidiophores are more or less erect, partitioned, with a series of branches that gives a characteristic structure in the form of brush producing long chains of conidia (Figure 4E).

Rhizopus stolonifer

Rhizopus stolonifer gives very invasive colonies on the surface of the medium. Morphologically, it presents a white thallus, filamentous and aerial (Figure 3F). At the microscopic level, many spores called sporangiospore are enclosed in sporangia (Figure 4F).

Mucor sp.

Mucor Sp. colonies are generally downy, invasive and greyish white to yellowish in color. Conidiophores are generally straight, entangled each in the other (Figure 3G). At their extremities, they give birth to a sporangium containing numerous conidia. Conidia are spherical, smooth and variable sizes (Figure 4G).

Fusarium nivale

Fusarium nivale colonies consist of a sparse mycelium, very tenuous, first white then turning yellow and finally brown (Figure 3H). Microscopically, there are no microconidia and the macroconidia are short, thick, hyalin, uniformly curved, one side being straight and the other curved. Conidia generally have 3 – 4 spetas (Figure 4H).

Distribution of isolated molds of peanut seeds sold in study markets

Figure 5 shows the diversity of isolated molds of different peanut samples collected in the

city of Douala. Several postharvest fungal isolates were grown in culture of which the most common genus are *Aspergillus* (52%), *Fusarium* (21%) and *Penicillium* (15%).

Geographical distribution of fungal genus

The distribution of the main type's genus of mold according to the main sampling sites is shown in Figure 6, from which all samples are found to be contaminated with mold and differ only in proportion. Thus in each of these markets, the most important genus is *Aspergillus* with the most important proportion to the Central market (68%) compared to 58 and 57% for the Dakar and Sandaga markets respectively

Distribution of fungal genus according to the peanut varieties recorded in the city of Douala

The figure 7 shows the distribution of fungal genus according to the varieties of peanuts sold in the markets of the city of Douala. It is noted that the most contaminated varieties are Garoua, Makmak and Yaounde. These by the molds dominance of the genus *Aspergillus*, *Fusarium* and *Penicillium* (Figure 7).

Despite the healthy appearance of peanut seeds sold in the city of Douala, they have been found to be contaminated by various fungal genera. This contamination is due to the initial charge in spores. Tahani *et al.*, (2008) which after incubation find the favorable medium for their germination and the formation of mycelia. This shows that reducing the water content of peanut seeds by drying before storage does not eliminate mold spores, but makes their development unfavorable. If the storage conditions are unfavorable, these molds are factors of bio deterioration affecting the quality of the

seeds. Furthermore, groundnut, by its high lipid and protein content, is a suitable medium that provides to the mold the nutrients needed for growth (Kassemi, 2006). Under the action of enzymes, complex polymers such as starch are transformed into simple molecules used by mold. Tabuc (2007), which could explain the high fungal density detected.

The genus *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizoctonia*, *Rhizopus* and *Mucor* represent the common mycoflora of all collected samples. Previous studies have shown that peanut seed storage molds in India included the genus *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Rhizoctonia*, *Stemphylium* and *Trichoderma*. Sud *et al.*, (2005). *Alternaria*, *Aspergillus*, *Fusarium*, *Moniliella*, *Penicillium* and *Rhizopus* genus were isolated from Algerian samples. Djabali (2012).

These results indicate that seed fungal contamination during storage may be related to hydrothermal conditions.(Wilson *et al.*, 2002) because the affected regions are subjected to completely different climatic conditions which would influence the fungal distribution. However, we can notice that the common fungal genera to all these regions are *Aspergillus*, *Fusarium* and *Penicillium*, with a predominance for the genus *Aspergillus* and *Penicillium*. Botton *et al.*, (1990) have indeed pointed out that during storage, a flora consisting of less cellulolytic and more osmophilic fungi are developing, which cause acidification of the substrate; these are mainly *Aspergillus* and *Penicillium* which are two genus that proliferate mainly during storage on substrates whose low humidity such as peanuts during storage and products with reduced water activity (Castegnaro and Pfohl Leszkowicz, 2002).

Fig.1 Peanut sampling (A, B, C, D, E represent Garoua, Makmak, Village, Yaounde and Bafia peanut respectively)



Fig.2 Varieties of peanut identified (A) and contaminant distribution during peanut storage (B)

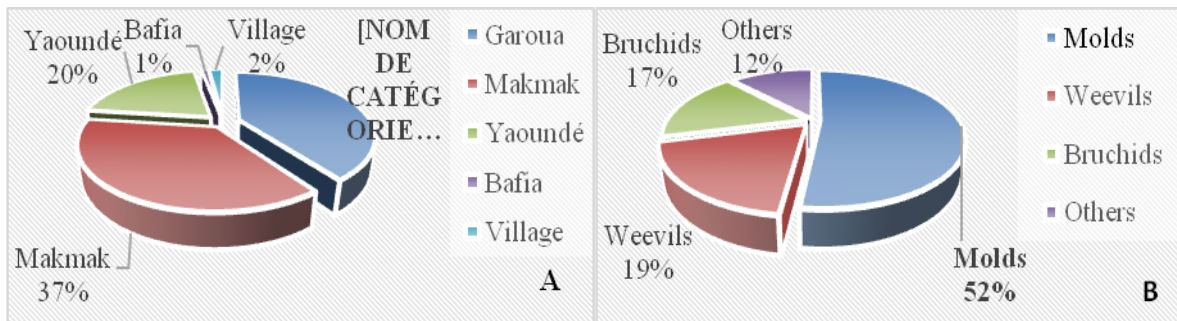


Fig.3 Aspect of different colonies of mold associated with the rotting of peanut seed (*Arachishypogaea*) during storage sold in the markets of Douala. A: *Aspergillus flavus*; B: *Aspergillus niger*; C: *Fusariumoxysparum*; D: *Rhizoctoniasolani*; E: *Penicillium*sp.; F: *Rhizopusstolonifer*; G: *Mucor* Sp. and H: *Fusariumnivale*

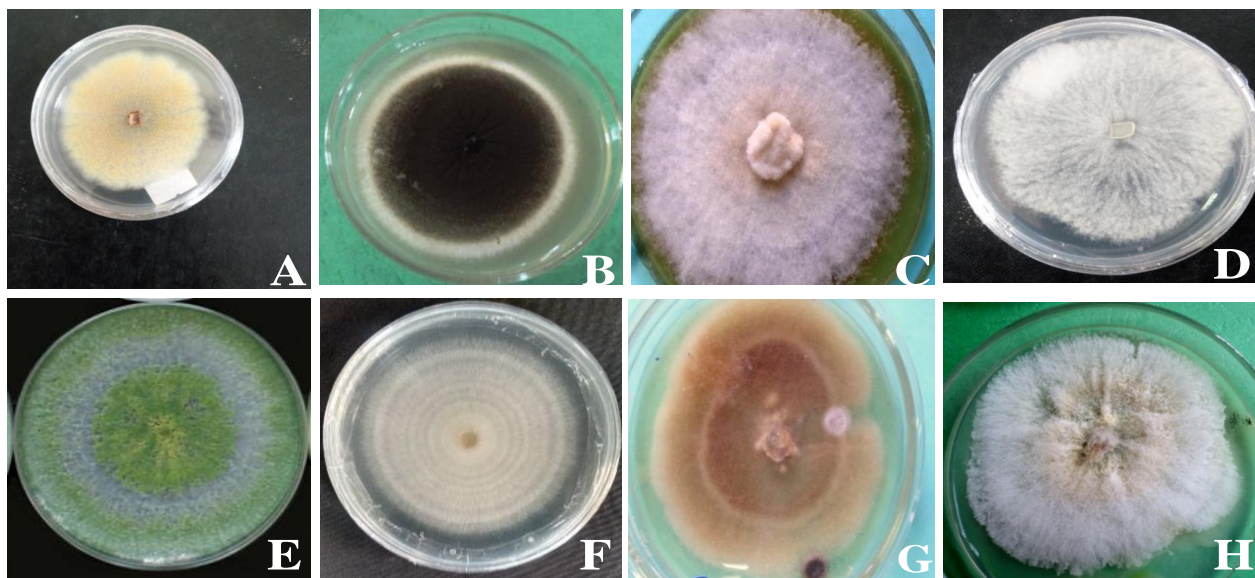


Fig.4 Microscopic observation of the main fungal species isolated from peanuts sold in Microconidia and macroconidia of *Fusarium oxysparum*; D: Mycelium of *Rhizoctonia solani*; E: Conidiophores producing long chains of conidia of *Penicillium sp*; F: Sporangiospore enclosed in sporangia of *Rhizopus stolonifer*; G: Conidiophores of *Mucor Sp.* and H: Microconidia and macroconidia of *Fusarium nivale*

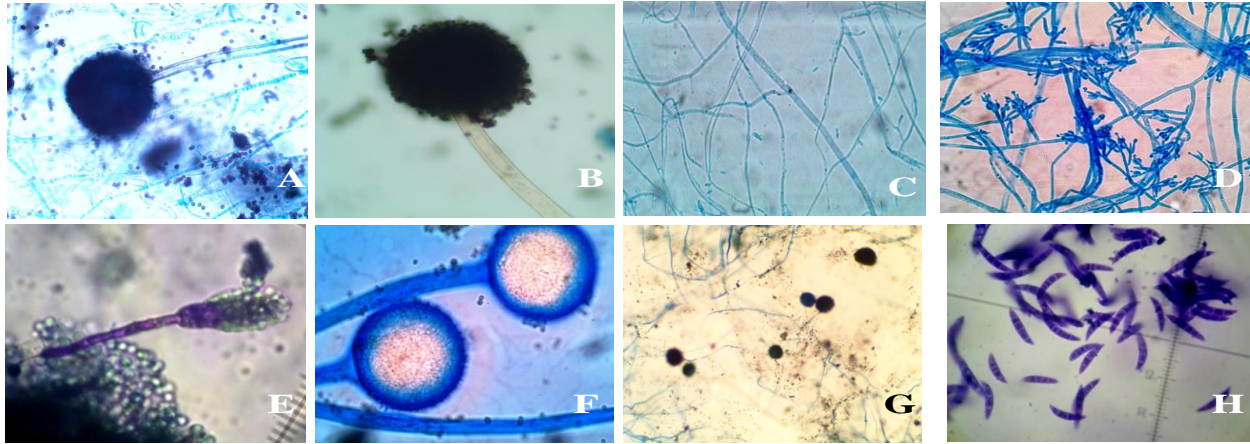


Fig.5 Diversity and proportion of fungal genus isolated from peanut samples in the city of Douala

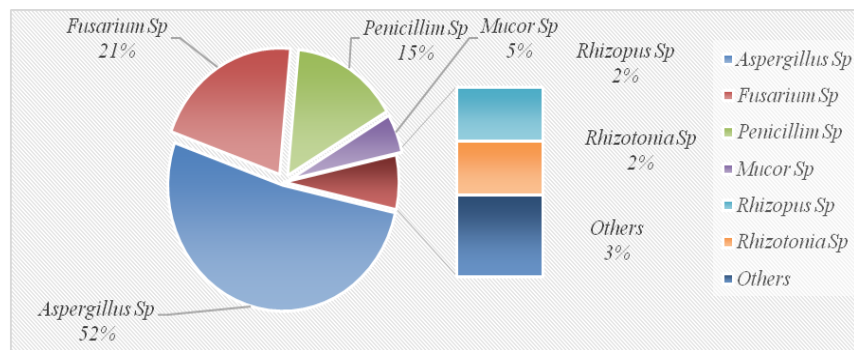


Fig.6 Diversity of different fungal genus according to markets

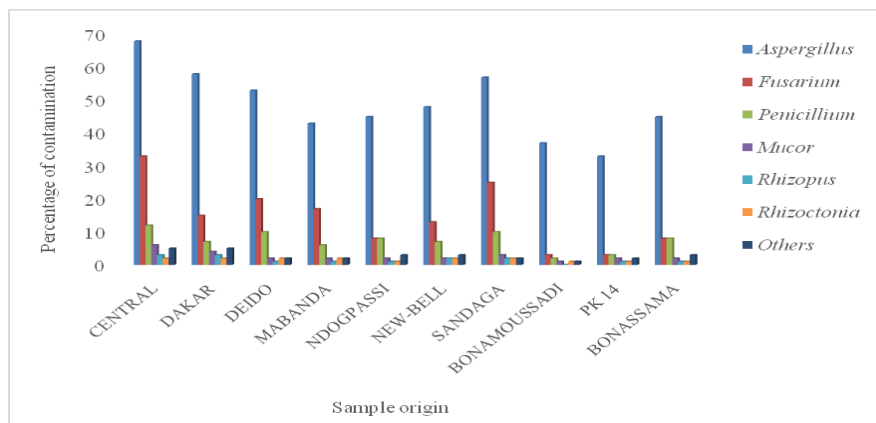
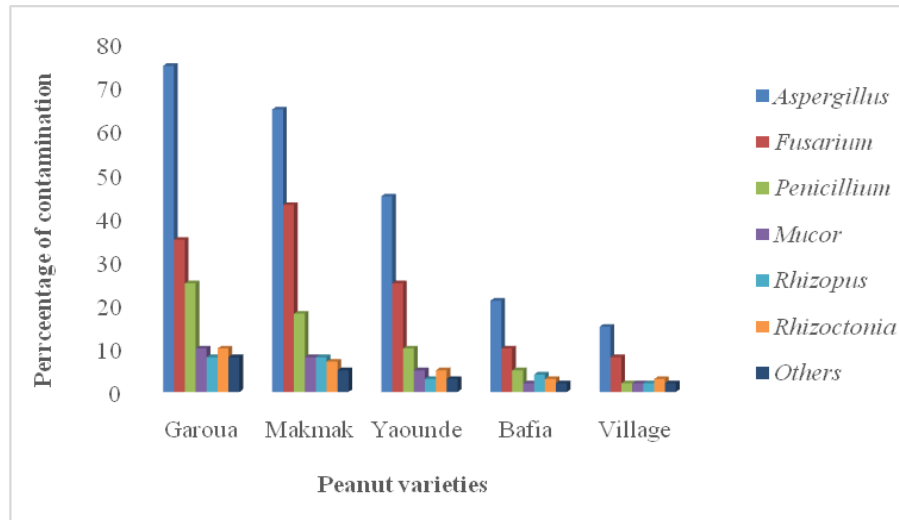


Fig.7 Distribution of fungal genus according to the peanut varieties recorded in the city of Douala



The highest contamination of peanut seeds by *Aspergillus* and *Penicillium* in all sampling sites indicates a potential danger because there is a possibility of a significant contamination of a aflatoxin and ochratoxin; similarly, the significant representativity of the genus *Fusarium* is also an element of concern because of the many mycotoxins that can be produced. In this study, peanut samples collected from different sites in the city of Douala are contaminated with identical mold species with some ready exceptions, but at different rates. This could be explained by the fact that these different sampling sites are submitted to the same climatic conditions. *A. Flavus* followed by *A. niger* were the predominant fungal species in all peanut varieties from the sampling sites. These results show that the quality of the fungal species present on seeds in stock is related to the peanut variety and also to the initial spore load. Therefore, there is a positive correlation between fungal infestation and seed richness in these two elements (Abdel Massih, 2007).

In conclusion, findings from our study showed that there are several contaminations of the peanut samples. These could be due to

the fact that postharvest fungal flora of peanuts in the city of Douala is composed of molds. However, further studies on molecular characterization to confirm their identification are needed.

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