



## **Diversity of Filamentous Molds Producing Mycotoxins in Rice Called "Deni Kachia" Sold on the Markets of Daloa (Côte d'Ivoire)**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### **ABSTRACT**

Filamentous fungi are frequent contaminants of many plant substrates and certain animal products. Their presence can improve the organoleptic quality of the product or alter it and lead to the accumulation of toxic secondary metabolites, such as mycotoxins. The objective of this work is to characterize the filamentous fungi isolated from the rice "deni kachia" stored and sold on public markets in the city of Daloa. A count and isolation of filamentous fungi, followed by macroscopic and microscopic identification was carried out. Several genera of filamentous fungi was identified. These are the genera *Aspergillus*, *Penicillium* and others. The different species of molds identified by conventional biochemical techniques were confirmed by the API 50 CH freezer. The genera *Aspergillus* and *Penicillium* are the most predominant in the samples analyzed. The presence of these species with their ability to produce mycotoxins suggests that contaminated rice poses a risk to the health of consumers.

**Keywords:** *Aspergillus*; *penicillium*; consumers; mycotoxins; rice.

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## 1. INTRODUCTION

Since the cultivation and storage of cereals and food, mold has caused deterioration. A fine white, black, green, orange, red and brown gradually invades food. These molds acidify, discolor, ferment and make these products unpleasant or even dangerous for consumption [1]. Until recently, mold was generally viewed as a simple unsightly deterioration of food, with the exception of *Claviceps purpurea*, which has been linked to human disease for 200 years. Japanese scientists highlighted the toxic nature of yellow rice 100 years ago. But it was not until 70 years later that the implication of mold was confirmed [1]. Until 1960 we did not realize that molds could produce significant toxins [2,3]. Molds are heterotrophic filamentous fungi, which not only have beneficial but also harmful effects on humans. Foods are generally very favorable environments for their development [4]. Several molds, notably the genera *Aspergillus*, *Penicillium* and *Fusarium*, are known to be contaminants of agricultural products and / or for their capacity to produce toxic secondary metabolites [5]. Cereals are staple foods for human and animal consumption worldwide. Rice is the third cereal produced in the world, for human consumption after wheat and corn. Its consumption per capita has passed 61, 5 kg / year in 1961 to 85.9 kg / year in 2002 [6,7]. World production has almost doubled in 20 years. Almost 90% is supplied by Asian countries, which control 70 % of the quantities sold on the international market [8]. In Côte d'Ivoire, as in many countries in West Africa, rice has become the main food for the majority of people. Due to strong urbanization and relatively high population growth estimated at 2.8%, rice consumption increased from 140.000 tons in 1961 to around 1.3 million tons in 2008 [9]. Despite the growing rice all over Ivorian territory, national production, estimated between 650 and 700.000 tons of rice equivalent milled in 2007, can only meet about half of the needs of domestic consumption. To make up for the deficit, Côte d'Ivoire uses rice imports mainly from Asia such as China, Thailand, Vietnam, Taiwan, at a total cost of more than 150 billion FCFA. As a result, Côte d'Ivoire is exposed to a risk of food insecurity on rice. General objective of this study is to characterize filamentous fungi isolated from "deni kachia" rice stored and sold on public markets in the town of Daloa. Specifically, this involves (i) counting the fungi in the rice sold on the Daloa markets, (ii) isolating the strains of filamentous molds counted in the

rice, (iii) identifying the strains of filamentous molds isolated from the rice "Kachia denial".

## 2. MATERIELS AND METHODS

### 2.1 Sampling

The samples consist of husks of rice, sold on the public markets of Daloa. They were collected at random, by purchase from public markets in the town of Daloa, then transported to the laboratory in a suitable container for analysis. Fifty "deni kachia" rice samples were collected from five sites, with 10 samples per site for this study.

### 2.2 Microbiological Analysis

A quantity of 10 g of rice grain was added to 90 ml of enriched broth, Buffered Peptone Water (EPT) (BioRad, Paris, France). The suspension was left at laboratory temperature for 30 minutes then decimal dilutions were made [10]. One milliliter of the desired dilution was inoculate into the petri dishes containing approximately 20 mL of sterile Sabouraud agar with chloramphenicol. The whole is well homogenized and the solidified dishes were incubated at 25 ° C for 5 days. The mushrooms was listed according to standard [11]. Two to four different colonies on the selected boxes were extracted and then introduced into Sabouraud broth (BioRad, Paris, France) for further work. One hundred and two of 102 strains were extracted.

#### 2.2.1 Isolation and identification of filamentous fungi

The isolation was carried out on the Sabouraud medium with chloramphenicol according to the work of [12,13]. Colonies selected on the different petri dishes were subcultured on Sabouraud medium with chloramphenicol (Biorad, Paris, France) and incubated at 37°C for 24 hours. These young colonies were used for identification, which was based on the analysis of macroscopic and microscopic morphological characters according to the work of [1]. Then a confirmation of the isolates was carried out by the API 50CH gallery.

## 3. RESULTS

### 3.1 Prevalence of Filamentous Fungi in Isolates

Macroscopic and microscopic examinations partially identified some types of filamentous

mold. Thus, out of one hundred and two (102) isolates, fifty three (53) were *Aspergillus* sp with a rate of 51.96%; 29 *Penicillium* sp, (28.43%) and 20 unidentified isolates with a rate of 19.60% (Table 1).

### 3.2 Diversity of Filamentous Fungi

Macroscopic examination coupled with microscopic examination made it possible to identify two genera of filamentous fungi, namely *Aspergillus* sp, *Penicillium* sp, and an unidentified group. The identification by the API 50 CH gallery made it possible not only to know the genera of the species identified from macro and microscopic examinations, but also to know the genus and species of unidentified isolates from macro and microscopic examinations (Table 2).

#### 3.2.1 Prevalence of filamentous fungal species in the rice samples analyzed

The identification with the API 50 CH gallery made it possible to know the prevalence of mold species in the rice samples analyzed. The predominant species were *Aspergillus niger* with a rate of 44% and *Aspergillus fumigatus* with a rate of 8% which were the weakest species (Table 3).

## 4. DISCUSSION

Analysis of the fungal flora of husked rice sold on public markets in Daloa has highlighted the presence of many different fungal species. The samples analyzed were found to be contaminated with mold with a prevalence of (51.96%) *Aspergillus*, (17.03%) *Penicillium*, (28.43%) and (19.60%) other unidentified fungi. The identification by the API 50 CH gallery made it possible to determine several species including *Eurotium* genus which was not identified by the conventional method. These results are comparable with the work of [14] who specifies that the main storage molds are *Aspergillus*, *Penicillium* and *Eurotium* (68%). Molds such as *Eurotium* (*E. chevalieri*, and *E. rubrum*) were detected but these are not dangerous [1, 15]. Cereals are the main vectors of mycotoxins. They can be contaminated, in the field or storage and the insects are the main vectors. The growth of toxigenic fungi, especially those producing aflatoxins, is the most favored in the countries with hot and humid climatic conditions, in particular the countries of Africa, South Asia and South America, Rice, corn and millet, the

staple food of people in these countries, were often contaminated with aflatoxins. Around 55 million tons of cereals are lost worldwide each year due to contamination [16, 17].

Penicillia levels are often high, presumably reflecting growth during the drying period. However, some species may have specific associations with particular cereals and are capable of developing before harvest. The most common species in wheat, barley and rice are Penicillia, particularly *P. citrinum*, *P. aurantiogriseum*, *P. chrysogenum*, *P. brevicompactum* and *P. crustosum* [18]. In general, cereals, corn and rice consumed in tropical regions were often contaminated with mycotoxins produced by molds of the genus *Aspergillus* or *Penicillium*. *Aspergillus* have a wide geographic distribution, but are more often associated with warm climate regions [19]; they develop on decomposing organic matter, in soil, compost, foodstuffs, cereals. Many species of *Aspergillus* are found in the human environment, particularly in dust and air [20].

Certain species can be directly pathogenic for humans and animals they are able to invade living tissue and cause aspergillosis, in particular, *Aspergillus fumigatus* responsible for pulmonary yeast infections; *Aspergillus niger* responsible for aspergillosis of the ear canal) [20,21]. 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 and in the biotechnology products industry, in particular for the production of enzymes and organic acids [22].

In our study, several species were identified at variable rates, these include *Aspergillus niger* (44%) *Aspergillus flavus* (26%), *Aspergillus candidus* (28%), *Aspergillus fumigatus* (8%), *Penicillium citrinum* (36 %), *Penicillium islandicum* (22%), *Eurotium rubrum* (14%) and *Eurotium chevalieri* (26%). According to the work of [1] in 139 Indonesian rice samples, the species most often found was *A. candidus*, present in 56% of samples, sometimes infecting up to 100% of grains, followed by *E. chevalieri*, *A. flavus*, *E. rubrum*, *A. fumigatus* and *A. niger*. In 73 samples of rice from the Philippines, *A. flavus* was the most common species present in 53% of samples, followed by *A. candidus*, *E. chevalieri*, *A. fumigatus* and *A. niger* [4].

**Table 1. Prevalence of filamentous fungi in isolates from rice samples from Daloa markets**

Sampled sites	Number of isolates	<i>Aspergillus sp</i>	<i>Penicillium sp</i>	Autres (ND)
Site I	22	11 (50.00 %)	6 (27.27 %)	4 (18.18 %)
Site II	23	12 (52.17 %)	7 (30.43 %)	4 (17.39 %)
Site III	18	9 (50.00 %)	5 (27.77 %)	4 (22.22 %)
Site IV	14	8 (57.14 %)	4 (28.57%)	3 (21.42 %)
Site V	25	13 (56.00 %)	7 (28.00 %)	5 (20.00 %)
Total	102	53 (51.96 %)	29 (28.43 %)	20 (19.60 %)

ND : Not determined

**Table 2. Identification of the species mold in rice “denial kachia”**

Number of isolates	Identification	
	Macroscopic et microscopic	API 50 CH
53	<i>Aspergillus sp</i>	- <i>Aspergillus niger</i> (22) - <i>Aspergillus flavus</i> (13) - <i>Aspergillus candidus</i> (14) - <i>Aspergillus fumigatus</i> (4)
29	<i>Penicillium sp</i>	- <i>Penicillium citrinum</i> (18) - <i>Penicillium islandicum</i> (11)
20	Autres (ND)	- <i>Eurotium rubrum</i> (7) - <i>Eurotium chavalieri</i> (13)

**Table 3. Prevalence of mold species according to rice samples**

Number of samples	Prevalence of identified mold species (%)							
	<i>A. niger</i>	<i>A. flavus</i>	<i>A. candidus</i>	<i>A. fumigatus.</i>	<i>P. islandicum</i>	<i>P. citrinum</i>	<i>E. rubrum</i>	<i>E. chavalieri</i>
50	22 (44%)	13 (26%)	14 (28%)	4 (8 %)	11 (22%)	18 (36%)	7 (14%)	13 (26%)

Penicillium species are responsible for keratomycosis (inflammation of the cornea), otomycosis (infection of the outer ear), onychomycosis (nail infection) and sometimes deep infections [23]. The most common causes of spoilage in dry cereals are *Eurotium* species, in particular *E. chevalieri*, *E. repens*, *E. rubrum*, *E. amstelodami*, and *Aspergillus penicillioides*. In rice and other small grains stored in tropical conditions, other subgenera of Penicillia, in particular *Biverticillium* and *Fusarium* are more common. *P. citrinum* is the most common *Penicillium* species in husked rice samples from Thailand, Indonesia and the Philippines. *P. islandicum* is present in 5% of samples from Indonesia, but was rarely detected in samples from the Philippines, and absent in rice from Thailand [24]. Many species belonging to the genus *Aspergillus* are known for their ability to produce certain mycotoxins [25]. *Aspergillus flavus* and *A. parasiticus* are the main producers of aflatoxins [26].

## 5. CONCLUSION

The identification of the pathogenic germs is essential to determine the possible means of action to limit the risk of contamination. The macroscopic and microscopic examinations and the contribution of the API 50 CH gallery made it possible to identify the isolated mycelial strains. Several species of filamentous mold in samples of rice sold on public markets in the town of Daloa are analysed in this study. This study has given us an idea of the diversity of microorganisms and species of filamentous fungi which are present in the rice samples of several genera. The presence of these molds is due to the poor storage conditions of the rice sold on the various markets in Daloa.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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