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Moulds infesting local and imported rice (*Oryza* spp) in Cameroon

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Abstract

Loss in quality and quantities of rice during storage is an important issue to focus on. Moulds contaminating rice were investigated and their injuries on rice during storage were evaluated. Local and imported rice samples sold in markets and mills were stored for 3 months under laboratory conditions. The contaminated grains were counted and analyzed to characterize storage moulds.

All rice samples evaluated were contaminated by moulds, right from sampling date. The quantity of mouldy grains varied from 1.1% for the rice sample from UNVDA to 4.2% rice brand 'Main dans la Main'. The highest mould infestation in terms of quality and quantity, was recorded on imported rice samples of world rice and 'Main dans la Main' 22.3 and 25.3% respectively; meanwhile 'Tox 3145 parboiled', Uncle Benz and Neima presented 7.5, 8.9 and8.9% respectively.

In general, imported rice samples contained the highest fungal load with a proportion of 65.9% compared to 34.3% for local samples. Among the 67 isolated strains, the genus *Aspergillus* dominated, followed by *Penicillium, Mucor* and *Circinella* with 13.4, 8.9, and 4.4% respectively. Therefore in Cameroon, some locally produced, but mostly some imported rice contain moulds from different genera, which damage rice at different proportions. It is urgent to develop methods to inhibit the growth of potential storage moulds and preserve the quality of rice consumed.

Keys words: Rice, contamination, storage, loss, quality.

1. Introduction

Rice (*Oryza*spp) is the third most widely cultivated cereal in the world after maize and wheat and with an estimate production of 430, 865 and 695mt respectively (FAOSTAT, 2012). Rice constitutes a staple food for half of the world's population. Today, rice is a commodity of strategic significance across many African countries (Hegde and Hegde, 2013), driven by changing food preference in the urban and rural areas and compounded by increased urbanization (Khalil *et al.,* 2009).

About 90% of world's rice is produced in Asia (Food and Agricultural Organization [FAO], 2015. To satisfy their increasing demand with a low production, African countries, particularly west and central Africa import large quantities of rice from Asia (Seck*et al*., 2010; Otsuka and Kijima 2010).

The physical quality of rice is determined by biophysical factors such as the agro-ecological zone of production and production system and by the production practices (Mapiemfu-Lamare 2017). Rice storage problems are very often caused by inadequacies during prior phases, particularly inadequate harvesting and drying (Saunders *et al*., 1978; Barnabas *et al*., 2008; Bala*et al*., 2010). Also, poor rice parboiling can lead to loss at storage (Diop*et al*., 1997; Fofana*et al*., 2011; Ogunbiyi, 2011; Ndindeng *et al*., 2014; 2015). Improper rice storage can lead to both quantitative and qualitative losses caused by pests, insects, rodents, sprouting, discoloration or contamination of grains with unwanted materials or substances. A large amount of rice is lost during storage, 1 - 100% of the total harvest (Hall, 1970; Schulten, 1975; Hopf*et al.,* 1976; Adams and Harman, 1977; De Padua, 1977; FAO and UNECA, 1977; Green, 1977; Harris and Lindblad, 1977; Mushi, 1978; Ren-Yong *et al*., 1990; IRRI, 1997; Appiah *et al*., 2011).

Hermetic storage in airtight bags significantly improves storage by protecting rice from rodents, insects and fungal infestation, but are not currently used in local mills or markets (Jones *et al*., 2011; Gitonga*et al*., 2013).

Grain losses may occur in storage due to moisture losses, rodents, insect infestation and fungal growth and subsequent price discounts for damaged grain (Kaminski and Christiaensen 2014; Kadjo*et al*., 2015; Kadjo*et al*., 2016). In addition, rice sold in markets or mills being it imported or locally produced are usually packaged in jute or plastic bags of 5, 10, 25 or 50 kg stored in inappropriate conditions; open to insects and rodents, high humidity etc. leading to the development of moulds.

This work studies moulds in order to estimate loss of the quality of rice grains locally produced and imported, during storage.

2. Material and Methods

2.1 Rice Samples

Plant material was rice grains: paddy grains or milled rice. Rice samples were collected from mills in Ndop rice development hub (RDH) in Cameroon and in the Mokolo market in Yaounde (a major urban consumption zone and the political capital of Cameroon). RDHs are zones (rice ecologies) where rice research outputs will be integrated across the rice value-chain to achieve the desired development outcomes and impact (Africa Rice, 2011). These samples were paddy and milled rice, imported from Thailand or locally produced and sold in Ndop hub (Table 1).

Rice samples were stored at room temperatures at the Institute of Agricultural Research for Development (IRAD) Yaoundé for 10 weeks. Data on physical quality were evaluated as follows.

The moisture content of rice grains was determined using a Satake Rice Moisture meter (Satake Co. Ltd., Tokyo, Japan) according to manufacturer's instructions and expressed as a percentage. The determination was done in triplicates.

Discolored rice grains were evaluated using a sample of 100 g of rice. Rice grains presenting any yellow, black or purple color, visualized under magnifying glass were manually selected from the normal grains and weighed. The evaluation was done every two weeks till the 10th week of storage to observe the evolution of discolored rice grains. Discolored rice grains were expressed as a percentage. The rice was held at an average relative humidity and temperature of 80% and 26°C respectively for 10 weeks, and samples taken weekly.

Table 1. Rice samples with respect to their origin and type

Rice variety or brand name	Origin	Type of rice
Main dans la Main (Mm)	Thailand	Milled w hite
Word rice (Wr)	Thailand	Milled w hite
Neima (Ne)	Thailand	Milled w hite
Uncle benz (Ub)	Thailand	Milled parboiled
Nerica L 56 (NE)	Ndop hub-Cameroon	Paddy
Parboiled paddy (Pp)	Ndop hub-Cameroon	Parboiled paddy
Tox 3145 (Tx)	Ndop hub-Cameroon	Paddy
Tox 3145 parboiled (Tx p)	Ndop hub-Cameroon	Parboiled paddy
Bamunka (Ba)	Ndop hub-Cameroon	Milled w hite
Jéhovah (Jé)	Ndop hub-Cameroon	Milled w hite
UNVDA (UN)	Ndop hub-Cameroon	Milled w hite
Ndop rice (Nd)	Ndop hub-Cameroon	Milled w hite

2.2. Characterization of Moulds

To isolate and obtain pure strains, PotatoDextrose Agar and Malt Extract Agar media were used as most of strains grow on semi-solid media.

The Ulster method which is direct and more indicative for the analysis of mould on food was used to detect, isolate and analyze mould present on the rice samples. In the Petri dishes containing moistened filter paper, 20 particles (discolored grains of rice) suspected to be contaminated were placed. The Petri dishes are then placed in a hermetically sealed plastic container in the dark and ventilated for 12 hours. The observation of the strains was carried out only after 5 to 7 days of incubation.

The transfer of strains was done under the laminar flow hood and consisted of aseptically transferring the isolated strain into new PDA and MEA culture media to perform the pure culture. Three (3) repetitions were performed for each strain. From the Petri dish containing the isolated strain, 0.6 cm of mycelium disk is transferred into new dishes containing the culture media. This operation is performed several times in order to obtain pure cultures. The sampling was preferably performed at the growth end of colonies. The Petri dishes were then placed at laboratory temperature (24-28 ° C).

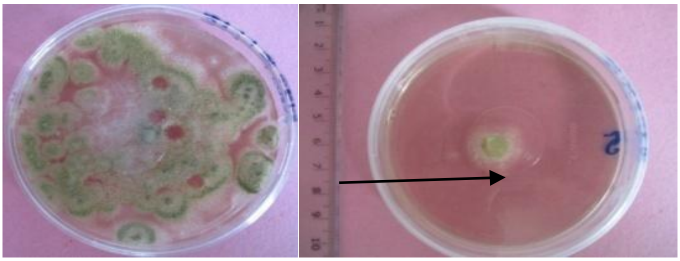

Fig. 1. Obtaining pure strain: a = isolated strain; b = pure strain (Douksouna photos, 2014)

The growth radius of mycelium was evaluated daily (48 hours after incubation) at the same hour. Each diameter is respectively measured on one of the two straight lines forming a right angle passing through the center of the explant (Fig. 2). The following formula was used to calculate the average growth radius.

$$D = \frac{d1 + d2}{2} - d0 \qquad \text{(Singh et } al.\text{, 1993)}$$

Where d0 is the diameter of the initial explant; d1 and d2 are the diameters of culture measured in both perpendicular directions.

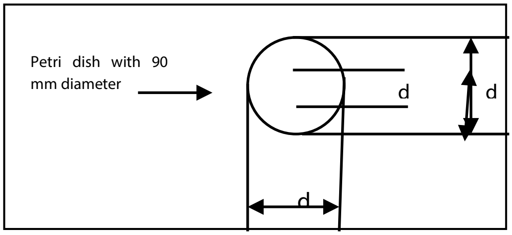

Fig. 2. Principle of measuring the radius growth of mycelium in Petri dish.
d0= diameter of the explant (0.7cm); d1and
d2=diameter perpendicular to pathogens.

The identification of many fungal species that can colonize food and alter the quality or even produce mycotoxins is an essential step in the evaluation of mycotoxic risk. A morphological identification was made based on the macroscopic characters (color, colony aspect, colony relief and the back of the boxes). The observation of the color and texture of the colony on the culture media as well as the microscopic structures made it possible to characterize the genera.

For all cultures obtained after 7 days, an identification key « Toxic Fungi in Food » allowed the characterization of moulds based on the technique of Pitt *et al*., (1997) according to the following characteristics:

- diameters of macroscopic colonies, measured in centimeter on the bottom of the box to evaluate growth;
- characters of colony, the appearance of the colony was observed by the naked eye under day light and in the presence or absence of a diffuse pigment to determine the colors of colonies.

Fungi were examined under the microscope as wet smears. To prepare a wet smear, a needle or an inoculation loop were used to collect a small portion of the colony with conidiogenous structures. The inoculum was taken from the edge of the colony because the fertile structures are young and the number of spores are acceptable. In addition, the structures that can enclose the spores have been taken near the center of the colony where the probability of finding mature spores is greatest.

The sample cut on a slide was first "wet" with a drop of ethanol 70% and a coverslip was laid, the excess liquid was blotted and followed by examination under microscope which is the microscopic study of the nature of differentiated organs. The observation was made at the objective 10 x and 20 x.

2.3. Data Analysis

The data obtained for all the parameters studied, were automated with the Excel software and the analysis were carried out by SPSS version 16.0. The different averages were compared at 5% significance level using Duncan's Multiple Range test.

3. Results and Discussion

3.1. Variation of Moisture Content of Rice Grains

There was a variation in moisture content among rice samples. In general, the level of moisture content was above 13% and could reach 15.6% at sampling. Moisture content (MC) is the weight of water contained in rice expressed in percentage. Moisture content is usually referred to the wet basis, meaning the total weight of grain including the water (IRRI, 2012). Results revealed a high moisture content at sampling date for imported milled rice samples sold at the Mokolo market: Uncle benz, Neima, World rice and Main dans la Main (Fig. 3); suggesting that these rice brands may be poorly imported and stored by rice traders. Ten weeks after storage at room temperature, the moisture content of all rice samples (except for Tox 3145 whose moisture content was 15.6% at sampling date and remained the same after storage) showed a linear increase; suggesting that storing rice grains at room temperature allows them to re-absorb moisture. On the other hand, moisture content increased only around one unit or even less for rice samples Nerica L56, Tox 3145, Main dans la Main (Fig. 3). These rice samples already had high moisture content at sampling date, around 14%, suggesting that once the moisture content of rice has dropped upon drying, it can increase by some units, but cannot easily increase above 17%. The rice grain is hygroscopic and responds dynamically and physically to moisture and temperature changes in the environment. A dry grain surface re-absorb moisture in a humid environment, while a wet surface desorbs moisture in a relatively dry environment. Moisture adsorption is associated with water reentering the grain. This occurs when the vapor pressure at the surface of a grain is lower than the vapor pressure in the surrounding air (Lan and Kunze, 1996).

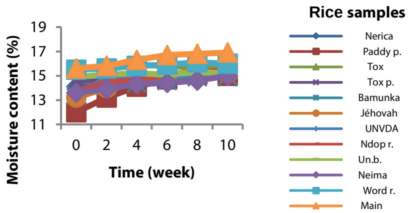

Fig. 3. Variation of moisture content of rice samples as a function of time

3.2. Discolored Rice Grains

Results revealed the presence of discolored rice grains in all the rice samples at the beginning of the experiment. The number of these discolored rice grains increased with time ($p < 0.05$), although at different frequencies within the different rice samples evaluated (Table 2).

All the four imported rice brands showed high number of discolored grains as compared with locally produced rice. Neima, Uncle benz, world rice and Main dans la Main which are the milled rice brands largely found in local markets in Cameroon like the Mokolo market where the sampling was done appeared to have highest number of discolored grains. This suggests that if these rice brands are stored at room temperature and humidity, there will be a large number of discolored grains, which are likely mouldy grain. These imported rice samples showed an increase of their moisture content during storage, meaning that when exposed to the ambient temperature and humidity, rice grains re-absorb moisture, creating favorable conditions for mould development, leading to discolored grains.

The rice brand which had the highest number of discolored grains was Main dans la Main at the beginning of the experimentand after 10 weeks storage. From my personal observations, this rice brand has a poorer appearance in the market and furthermore, its price is always lower than other imported rice brands sold in the market.

Table 2. Evolution of discolored rice grains in imported and local rice with respect to time

Time (week)	Rice brand										
	Nerica L56	Parboiled paddy	Tox 3145	Tox 3145 parboiled	Bamunka	Jéhovah	UNVDA	Ndop rice	Uncle benz	Neima	World rice	Main dans la Main
0	21a	25a	29a	36a	09a	11a	11a	17a	29a	19a	75a	117a
2	30a	49a	44a	107b	14a	14a	17a	25a	60a	46a	146b	170b
4	41a	53a	53a	163c	41ab	41ab	41a	31a	151b	57ab	203c	231c
6	53ab	63ab	59a	167c	45ab	43ab	43ab	38a	225c	68ab	235d	372d
8	57ab	67ab	63ab	173c	46ab	49ab	46ab	42a	231c	74ab	243d	385d
10	61ab	78ab	81ab	178c	49ab	57ab	49ab	53ab	237c	87ab	257d	389d

Means in a column with the same letter are not significantly different at $p < 0.05$ (Duncan's Multiple Range test).

The evaluation of discolored grains in terms of percentage showed that the mean total percentage of discolored rice grains 10 weeks after storage was high in imported rice brand and inTox 3145 parboiled (Fig. 4). The level of discolored rice was equal or higher than 3% in these rice samples, meaning that theycannot be graded premium or grade 1 rice, according to the Quality Standard for milled rice in the Philippines for instance.

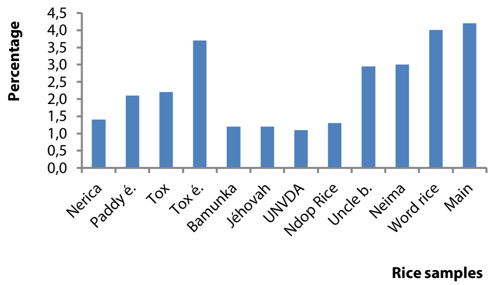

Fig. 4. Percentage of discolored rice grains in per rice brand or samples 10 weeks after storage

Percentages with the same letter are not significantly different at $p < 0.05$ (Duncan's Multiple Range test).

3.3. Characterization of moulds

3.3.1. Description of the isolated strains

The analysis of rice mycoflora revealed several genera distinguished by their morphological characters (Pitt et al., 1997).

The genus *Aspergillus* with seven species isolated: *Aspergillus niger*, *Aspergillus flavus, Apergillus ochraceus, Aspergillus fumigatus, Aspergillus oryzae, Aspergillus japonicas, Aspergillus parasiticus*. The genus *Penicillium,* the genus *Mucor* and the genus *Circinella.*

3.3.2. Characterization of moulds

Isolated colonies were keyed to species using Pitt *et al*., (1997). Results showed different ($p < 0.05$) growth of fungi according to their virulence. The radius growth of all the fungi increased with time from the second day to the fifth day, this at different growth rate (Table 3). The most virulent fungus was *Mucor* sp, whose growth radius was rapid and reached 7.4 cm at day five, followed by *Circinella* sp, *Aspergillus parasiticus* and *A. orchraceus* whose growth was comparable. The slowest growth radius was observed with *A. niger* (Table 3).

Table 3. Radius growth (cm) rate of fungi

An: Aspergillus niger; Af: Aspergillus flavus; Aj: Aspergillus japonicus; Afu: Aspergillus fumigatus; P: Penicillium; Aor: Aspergillus orchraceus;C:Circinella; Ap: Aspergillus parasiticus; M: Mucor.

Time (day)	Fungi									
	An	Ao	Af	Aj	Afu	P	Aor	C	Ap	M
2	2.5c 1	1.9a 1	3.0de 1	2.5c 1	1.3a 1	2.9de 1	2.9de 1	3.0de 1	3.0de 1	3.4f *1*
3	2.8a 1	2.5a 1	4.0c 12	3.6b 1	2.3a 1	4.0c 1	4.1c 1	4.1c 1	4.2c 1	4.7d *1*
4	3.1a 1	3.2a 1	5.0cd 2	4.7b 1	3.0a 1	5.4de 2	5.3d 2	5.3d 1	5.4e 1	6.0f *2*
5	3.4a 1	4.3b 2	6.0de 23	5.8c 2	3.7b 1	6.2de 2	6.5f 2	7.0f 2	6.6f 2	7.4g *2*

Figures on a line with the same letter are not significantly different at $p < 0.05$; figures in bold and italic represent the comparison in column (Duncan's Multiple Range test).

3.4. Virulence of strains on rice samples

Mycological analysis showed that imported rice samples of world rice and Main dans la Main presented the highest fungal load (Table 4). No strain was isolated on Nerica L56. On world rice and

Main dans la Main rice samples, were isolated respectively eight and five different strains. This result corroborates previous ones obtained in the course of this study, where the rice brands world rice and Main dans la Main presented high moisture content and discolored grains; suggesting that rice grains with high moisture content and discolored grains probably possessed a high fungi load.

Table 4. Identified fungi per rice sample

Fungi	Rice sample											
	NE	Pa	Tx	Tx. P	Ba	Jé	UN	Nd	Ub	Ne	Wr	Mm
A. flavus	-	-	-	-	x	-	x	-	-	-	x	x
A.fumigatus	-	-	-	-	-	-	-	-	-	-	x	x
A. japonicus	-	-	-	-	-	-	-	x	-	-	x	x
A.niger	-	x	-	-	-	x	-	-	-	x	-	-
A. ochraceus	-	-	-	-	-	-	-	-	x	-	x	-
A. oryzae	-	-	-	-	-	-	-	x	-	-	x	x
A. parasiticus	-	-	-	x	-	-	x	-	-	-	x	x
Penicillium sp	-	-	-	x	x	-	-	x	x	-	-	-
Mucor sp	-	-	x	-	-	-	-	-	x	x	-	-
Circinella sp	-	x	-	-	-	-	-	-	-	-	x	-
Others	-	-	-	x	-	x	-	-	x	x	x	-
Total Number (67)	-	2	1	3	2	2	2	3	4	3	8	5

- Means absence; x stands for presence. Main dans la Main (Mm), Word rice (Wr), Neima (Ne), Unclebenz (Ub), Nerica L 56 (NE), Parboiled paddy (Pa), Tox non étuvé (Tx), Tox parboiled (Tx. P), Bamunka (Ba), Jéhovah (Jé), UNVDA (UN), Ndoprice (Nd).

In terms of percentage, the genus *Aspergillus* dominates with a total of 63.8% as compared to the other strains. The genus *Penicillium*, *Mucor*and *Circinella* presented respectively 13.4, 8.9 and 4.4% (Fig. 15).

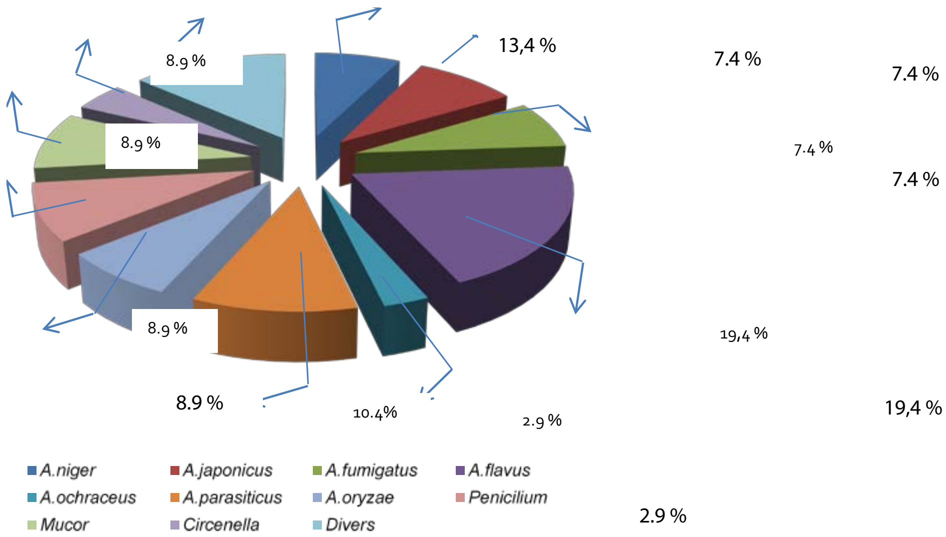

Fig. 5. Proportion (in percentage) of isolated strains infecting rice samples in Cameroon

4. Conclusions

This study evaluated some rice samples in Yaounde (Mokolo market) and Ndop rice development hub, which are respectively the main market of the political capital of Cameroon (Yaounde) and a rice-production division. There were imported milled rice brands sold at the Mokolo market and paddy or milled rice brands produced in Ndop.

It was found that the different rice samples were contaminated by moulds; certainly because of their high level of moisture content. The fungal charge was high in imported rice sample brands as compared to locally produced rice, suggesting that these rice samples may be imported or stored and sold in poor conditions, leading to the development of moulds.

In addition, isolated mycoflora was diverse. Four genera were found: *Aspergillus*, *Penicillium, Mucor*and *Circinella*. *Aspergillus* species dominate followed by *Penicillium* and *Mucor*.

Further studies can take into consideration all imported rice brands and rice samples produced locally and sold in local markets in Cameroon and assess the presence of toxin like aflatoxin in the rice grains.

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Reduction of fungi and mycotoxin decontamination by ozone gas treatment in three stored rice (*Oryza sativa* L.) varieties

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Abstract

The present work brings together different rice varieties (black, brown and white) evaluated for their differences/ susceptibilities/resistance to ozone (O3) gas treatment for safer storage (mycological and toxicological contamination control). The three rice varieties were separated into two Groups –Control (GC) andtreated Groups (GT) which had O3 gas applied (5 L/min, 40 ppm and 60 min for gas flow). Samples were collected during the storage period to check for the O3 gas effect on fungi reduction (total count and fungi genera identification) and so for the humidity parameters of moisture content (mc) and water activity (aw). It was possible to verify the effectiveness of the O3 application in the samples when compared to Control. It was observed that even at the shortest time of gas exposure, O3 application caused changes to fungi (both growth speed & toxin formation). The grains did not change their organoleptic, physical and biochemical characteristics after O3 application. Recent studies from our Labmico Group indicated that the O3 application in addition to prevention of the biological contaminants, as reported in the current work, also reduces an insecticide (deltamethrin) residues. As O3 treated grain has reduced fungi contamination and toxicity of rice grains in all the varieties studied, it can be considered a potential agent to control fungi spoilage and so for toxigenic strains. Considering that there is a growing concern on the use of agrochemicals and their harmful effects on human health and the environment, O3 application can be a promising to implement decontaminationof highly consumed grains worldwide, such as rice.