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Mycological analysis of rice from stores in Igbemo-Ekiti (a rice producing area) of Ekiti State, Nigeria

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ABSTRACT

Rice (*Oryza sativa* L.) is the most common staple food consumed in Nigeria. For health reasons people now prefer to consume the local unpolished rice over the processed foreign ones. Igbemo-Ekiti is a town that produces rice in Ekiti state and the rice is very popular amongst inhabitants of the state and beyond. The storage facilities in the town are still traditional. This work was undertaken to assess the mycological quality of stored rice from Igbemo in order to ascertain the microbiological safety. Rice samples were aseptically collected into dry sterile sample bottles from ten rice store houses in Igbemo-Ekiti (a rice producing locality) in Ekiti State Nigeria. The samples were transported to the laboratory immediately they were collected and stored at 4°C until required for analysis. Physical observations were carried out on the samples for obvious growth of moulds such as spotting, caking, discoloration and odour. The rice samples were cultured on Potato Dextrose Agar (PDA) using the pour plate and the seed distribution methods after surface sterilization in 1% hypochlorite solution. The plates were incubated at 25°C for 5 to 7 days. The result obtained showed that the major fungi isolated from stored rice samples from Igbemo Ekiti were *Aspergillus niger* (10%), *Aspergillus flavus* (10%), *Aspergillus fumigatus* (70%), *Botrytis cinerea* (20%), *Fusarium* sp. (10%), *Mucor mucedo* (90%), *Penicillium citrinum* (40%), *Rhizopus nigricans* (10%) and *Trichoderma viride* (10%). The fungal counts on the rice samples were found to range from 1.0×10^2 to 7.0×10^5 sfu/g. It was concluded that since some of these fungi could be mycotoxigenic; there is need for a control programme for the organisms in stored rice in the study area. It was therefore recommended that storage conditions of the stores should be improved by the aid of modern storage facilities and cleanliness should be observed by frequent cleaning and fumigation of these store houses. Enlightenment campaign is necessary for the farmers and traders who handle Igbemo rice so that they can understand the consequences of mould growth on the stored rice.

Keywords: Mycological, rice, stores, Igbemo-Ekiti, fungi.

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INTRODUCTION

Rice is the seed of the grass family *Oryza sativa (*Asian type) or *Oryza glaberrima* (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. It is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize (FAOSTAT, 2012).

In the tropics, rice is the primary source of human nutrition, and is one of the cheapest sources of food energy and protein (Mejia, 2003).

Rice constitutes the staple food for most Nigerian families. The rice mostly consumed is polished and is imported from Thailand and other rice producing countries of the world this is because it is easier to cook because it is cleaned up unlike the local variety where you have to first try and pick the stones and blow off the chaff before cooking thereby constituting too much labour for house wives and other rice consumers. In recent times however, Nigerians have started turning to consume rice that is produced locally for the health

benefits associated with consuming unpolished rice. Again the government has placed a ban on imported rice so if available, the cost is prohibitive.

Brown or unpolished rice is rich in Manganese and essential nutrients like Magnesium and Selenium so is said to prevent Artheriosclerosis, reduce risks of colon cancer. Due to its rich mineral content, brown rice is good for hair, teeth and nails (Radhakrishna, 2010).

Food contamination is a worldwide problem and spoilage fungi have since been recognized as major source of contamination. Contamination of various commodities by fungi and the hazard of consuming the contaminated products have been noted worldwide (Pitt and Hocking, 2009). Xerophilic fungi like *A. candidus* and *Eurotium* spp. were predominantly isolated from rice grains sent from Pakistan to Uganda (Taligoola et al., 2011). Most of these fungi which contaminate produce are known to produce mycotoxins. Ashiq (2015) noted that several studies have revealed mycotoxin contamination in rice worldwide e.g aflatoxins have been reported in the United Arab Emirates (Osman et al., 1999), fumonisins in Iran (Alizadeh et al., 2012), OTA in Morocco (Juan et al., 2008a), ZEA in Nigeria (Makun et al., 2007), DON in Italy (Lorè et al., 2011), nivalenol in Korea (Lee et al., 2011), sterigmatocystin in Japan (Takahashi et al., 1984), and citrinin in Egypt (Abd Alla, 1996).

Igbemo-Ekiti is a rice producing town in Southwest Nigeria. Farmers here carry out their farming activities using crude means as there are no machines and other labour saving devices. The storage facilities for harvested rice are also crude so rice is stored in jute bags in poorly ventilated structures. The present work therefore is aimed at assessing the mycological quality of rice obtained from the storage houses in the town under study.

MATERIALS AND METHODS

Description of sample area

Igbemo-Ekiti is a traditionally prosperous agricultural town in Ekiti State, Southwest Nigeria. The town is popularly called the home of rice. It is located on altitude 461 m, latitude 7° 42' 0.0"N and longitude 5° 20' 60.00"E. In Ekiti State, agriculture is the mainstay of the people so it provides income and employment for more than 75% of the population. The state enjoys a conducive climate so there is luxuriant vegetation.

Collection of samples

Rice samples were collected asceptically from rice bags in the ten store houses visited using a sterile scoop with a sharp end to pierce each bag to be sampled. On being pierced, rice grains were delivered into the scoop. The samples were put into appropriately labeled sterile sample bottles and taken to the laboratory for analysis. In the laboratory, the samples were stored in the refrigerator at 4°C until needed for work.

Physical examination of rice samples

The rice samples were examined for obvious signs of spoilage such as spotting, discolorations (using naked eyes) and sniffing to check for off odour.

Isolation of fungi

Seeds were surface sterilized by immersing in 1% sodium hypochlorite for 2 min and rinsed in several changes of sterile distilled water. The surface sterilized seeds were placed on malt extract agar, potato dextrose agar, Sabouraud dextrose and yeast-malt extract agar. To each of the plates 50 mg/L chloramphenicol was added (Lugauskas, 2005). All plates were incubated at 25°C for 5 to 7 day. Subcultures were made on sterile media plates and incubated appropriately.

The identification of the isolated fungi was done both macroscopically and microscopically. The gross morphology of the fungal growth on plates was studied including their colors. Later small portions of the fungal pure culture were teased and mounted in lactophenol in cotton blue dye on a clean slide, covered with a clean cover slip and observed under the microscope (Fawole and Osho, 1995). The identity of the fungi were certified using cultural characteristics and pathogenicity tests as well as comparing them with confirmed representatives identified by means of keys as outlined in Barnett and Hunter (1998).

Determination of % occurrence of the fungal isolates

This was done to determine the incidence of occurrence of the different fungal isolates. The total number of each isolate in all samples was obtained against the total number of all the isolates in all the samples screened. Frequency of occurrence was therefore determined using method described by Giridher and Ready (1997).

$$\text{Percentage of frequency} = \frac{\text{No. of observations in which a species appeared}}{\text{Total no. of observations}} \times 100$$

RESULTS

The result obtained showed that fungi were isolated from all the rice samples analyzed (Tables 1 and 2). In all the samples there were visible signs of discoloration and rusty odour. Running a hand into the rice bag showed that dust particles covered the hand. Figures 1 to 3 show the rice stores and figure 4 shows the mill for rice in Igbemo-Ekiti. The fungi isolated and their frequencies of occurrence were: *Aspergillus niger* (10%), *Aspergillus flavus* (10%), *Aspergillus fumigatus* (70%), *Botrytis cinerea* (20%), *Fusarium* sp. (10%), *Mucor mucedo*

Table 1. Fungi isolated from rice from various stores in Igbemo-Ekiti.

Store code	Fungi Isolated
A	*Aspergillus flavus*, *Aspergillus niger*, *Mucor mucedo*
B	*Aspergillus fumigatus, Penicillum citrinum, Mucor mucedo*
C	*Aspergillus fumigatus, Penicillum citrinum*
D	*Aspergillus fumigatus, Rhizopus nigricans*, *Botrytis cinerea*
E	*Mucor mucedo*
F	*Aspergillus fumigatus Penicillum citrinum, Mucor mucedo*
G	*Aspergillus fumigatus*, *Mucor mucedo, Botrytis cinerea*
H	*Aspergillus fumigatus*, *Mucor mucedo, Fusarium* sp.
I	*Penicillum citrinum, Mucor mucedo*
J	*Mucor mucedo, Trichoderma viride*

Table 2. Frequency occurrence of Fungal isolates from rice in various stores.

Fungi isolated from rice from different stores	Frequency of occurrence of fungal isolates	% frequency of occurrence of fungal isolates
Rhizopus nigricans	1	10
Mucor mucedo	9	90
Aspergillus flavus	1	10
Penicillium citrinum	4	40
Aspergillus niger	1	10
Aspergillus fumigatus	7	70
Fusarium sp.	1	10
Trichoderma viride	1	10
Botrytis cinerea	2	20

Figure 1. One of the stores for rice.

(90%), *Penicillium citrinum* (40%), *Rhizopus nigricans* (10%) and *Trichoderma viride* (10%) (Table 1). The fungal counts on the rice samples were found to range from 1.0×10^2 to 7.0×10^5 sfu/g (Table 3). The least

Figure 2. Rice packed for sale in one of the stores in Igbemo-Ekiti.

Figure 3. Modern rice store recently built in Igbemo-Ekiti.

Figure 4. The mill for rice in Igbemo-Ekiti.

Table 3. Fungal counts on rice from the Igbemo rice stores.

Store code	Fungal counts
A	5.0×10^5
B	7.0×10^3
C	5.0×10^2
D	3.0×10^5
E	2.0×10^2
F	1.0×10^5
G	7.0×10^5
H	3.0×10^3
I	2.0×10^5.
J	3.0×10^5.

count being in store E and the highest being in store G.

DISCUSSION

These fungi isolated which are similar to the ones isolated by Makun et al. (2007) must have gotten to the rice samples from the mills or from new supplies being stored in old contaminated containers. The isolates are majorly storage fungi which are similar to those isolated by Begum and Samaipathi (2000), Aydin et al. (2011) and Sakai et al. (2005) also reported the presence of similar fungi in stored rice. This similarity is not far-fetched because due to high humidity and temperature, crops in tropical region are more susceptible to mold growth and mycotoxins contamination than those in cooler regions (Wagacha and Muthomi, 2008). The presence of fungi in the grains can lead to losses in quality (Jayaraman et al., 2011) because they also derive nutrients from the food which they grow on. These fungi isolated are also associated with the soil so could have come into the store from farm. They can be eliminated via proper farm hygiene and good agricultural practice. Most have also been associated with street vended foods; for instance Ezekiel et al. (2012) isolated Aflatoxigenic moulds from street vended snacks in Lagos, Nigeria. When they eventually get in to the food they can lead to food spoilage or produce toxins which are taken in along with the food.

The rice mills are also likely sources of these fungi as the same mill used for milling contaminated samples can easily cross contaminate samples that were free before being taken to the mill. The storage facility which has poor temperature and moisture control is also implicated in the transfer of fungi as the houses are crude with poor ventilation and lighting. Atanda et al. (2011) reported that the principal factors governing fungal growth are water activity, temperature, gas tension and preservatives (including fungicides and fumigants). The growth of fungi can be controlled but are not considered in the rice stores visited hence the isolation of theses fungi from rice. The rice bags are kept on the ground with one bag stacked on another which is another method by which cross contamination can occur. The presence of these fungi species suggests a potential for mycotoxin production as suggested by Tonon et al. (1997).

Prompt, proper drying is the best means to avoid fungal growth and mycotoxin production in grain after harvest. At times when sun drying is not possible or unreliable some form of mechanical drying may be necessary (FAO, nd).

CONCLUSION AND RECOMMENDATIONS

Some of the fungi isolated are known producers of mycotoxin so the control of the fungi to prevent occurrence of mycotoxin in the rice samples is necessary. There is therefore need for a programme to be put in place for the control of the organisms in stored rice in the study area. Conditions in the store houses should be improved to discourage mould growth by the aid of modern storage facilities and cleanliness should be observed by frequent cleaning and fumigation of these store houses. Enlightenment campaign is also necessary for the farmers and traders who handle Igbemo rice so that they can understand the consequences of mould growth on the stored rice. The production of rice should not be left in the hands of the peasant farmers alone rather the government should invest in provision of modern rice production equipment and facilities as this will ensure that only healthy and safe foods get to the populace and also help give economic benefit to the farmer.

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