Nutritional Sustainability for a Child towards Isolation of *Aspergillus* Species from Some Cereals and Legumes Sold in Enugu

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Abstract

Background of the Study: Most of Nigerian staple foods especially cereals (rice and beans) are liable to aflatoxin contamination due to inherent high temperature and humidity favouring the growth of aflatoxin-producing *Aspergillus* spp.

Objective: This study was therefore conducted to determine the rate of contamination of some rice and beans varieties sold in Enugu state.

Methodology: A total of 220 samples were randomly purchased from various markets in Enugu state and analysed using standard mycological techniques.

Results: Of the total of 243 fungal isolates the most predominant were *Aspergillus* spp 106 (43.6%) with more numbers in rice varieties 84(50.3%) than beans varieties 22(28.9%). This is followed by *Rhizopus* spp 88(36.2%).

Statistical Analysis: All generated data were subjected to statistical analysis using SPSS version 24. Confidence interval was recorded at $P < 0.05$ limit.molars.

Conclusion: This study showed that rice and beans sold in Enugu were contaminated by fungal agents irrespective of the variety and some of these fungal agents and their metabolites (aflatoxins) pose a serious health hazard either through consumption or inhalation.

Keywords: Rice; Cowpeas; Aflatoxins

Introduction

Cowpea (*Vigna unguiculata* (L) Walp) like other legumes is an important foodstuff in tropical and subtropical countries. It is widely grown cultivated and distributed in Africa, Asia, West Indies, Latin America and India. The legume can be grown in marginal soils and in arid or semi-arid regions. Its deep penetrating root systems enable it to withstand very dry conditions. The nutritional value of about 25% protein is highly valuable where many people cannot afford animal protein [1] especially in children. The protein have well recognized deficiency of the essential sulphur-bearing amino-acids, methionine and cystine [2].

Rice (*Oryza sativa* Linn.) is a wholesome cereal grain that is ideal for diverse nutritional needs. It contains predominantly carbohydrates besides vitamins and fibre and has higher proportion

of protein than in wheat, corn and sorghum [3]. It has become the predominant staple food in about 33 countries of the world including Nigeria [4]. About 90 - 95% of Nigerians consume rice and this cut across all economic class where it is eaten in different recipes [5]. Despite Nigerian being the highest producer of rice in West Africa the yield in Nigerian is low compared to other parts of the world. This limited rice and cowpea produced is further reduced during post-harvest storage [6] due to the prevailing high temperature and relative humidity which encourage colonization of moulds. Moulds have been found to contaminate maize [7], wheat [8] and rice [9-11]. The presence of moulds in stored grains may lead to various forms of deterioration, visible mouldiness, seed discoloration [12] and decreased nutritive value [13]. However, the most debilitating effect of mould deterioration of stored grain and seeds is mycotoxin production [14]. Mycotoxins are important in

food safety in that they not only lead to deleterious health effects in humans and animals but also pose adverse economic implications especially the aflatoxins caused by some species of *Aspergillus* [15,16].

Aflatoxin is about the most popular and widespread mycotoxin [16]. They are secondary metabolites produced by the species of *Aspergillus* that contaminate a variety of agricultural food commodities [15]. They are recognized to be hepatotoxins and carcinogens for humans [17]. *Aspergillus* and *Penicillins* produce their toxins mostly in stored seeds, hay or commercially processed foods and feeds although infection of seeds usually takes place in the field [18]. Several disease outbreaks of aflatoxicosis in humans and animals have been reported due to the consumption of aflatoxin contaminated food and feed [19].

In Nigeria rice and cowpea (beans) are used for a variety of food and nonfood products (snacks) and they are some of the important cereals which favour aflatoxin production [20]. Both the toxigenic fungi and the secondary metabolites they produce are potential problems in both health and economic perspectives. Also fumes from burning molded hay may also affect animals and man handling of such hay thereby causing in them dermatitis and conjunctivities.

Objective of the Study

The objective of this study is to isolate some species of *Aspergillus* and other fungi associated with rice and beans as part of information towards formulating control regimens against the dreaded mycotoxins in this part of the world.

Methodology

Sample collection

A total of 220 samples were randomly selected from rice and beans varieties from different markets in Enugu state. These samples include exposed and unexposed samples. The research work was carried out between June and July 2019 in Enugu.

Culture of samples

Samples of different varieties of rice and beans were surface sterilized using 0.1% sodium hypochlorite and then rinsed in two changes of sterile water. They were separately inoculated on a Sabouraud Dextrose agar slants supplemented with 50 mcg/l of chloramphenicol and incubated for two weeks. All tubes showing significant growth were examined and identified according to Frazier WC and Westhoff DC [21].

Statistical analysis

This was done using Turkey’s multiple tests and the student’s t- Test SPSS version 24. There was statistically significant difference between the *Aspergillus spp* isolates in rice and beans varieties (P < 0.05). No statistically significant differences were observed among the exposed beans varieties and rice varieties (P > 0.05) in the rate of other fungal isolates whereas a statistically significant

variation was observed for the unexposed samples of rice varieties and beans varieties studied respectively (P < 0.05).

Results

Fungal Isolates	Rice	Cowpea
<i>Rhizopus spp</i>	48 (28.7%)	40 (52.6%)
<i>Aspergillus niger</i>	44 (26.3%)	12 (15.8%)
<i>Aspergillus flavus</i>	18 (10.8%)	8 (10.5%)
<i>T. mentagrophytes</i>	15 (9.0%)	3 (3.9%)
<i>Aspergillus fumigatus</i>	13 (7.8%)	2 (2.6%)
<i>Mucor spp</i>	9 (5.4%)	-
<i>Geotrichum spp</i>	8 (4.8%)	-
<i>Aspergillus. tamarri</i>	3 (1.8%)	-
<i>Synephalastrum spp</i>	2 (1.2%)	8 (10.5%)
<i>Aspergillus clavatus</i>	2 (1.2%)	-
<i>Aspergillus restrictus</i>	2 (1.2%)	-
<i>T. rubrum</i>	1 (0.6%)	-
<i>Aspergillus caesiellus</i>	1 (0.6%)	-
<i>Microsporum canis</i>	-	2 (2.6%)
<i>Scopulariopsis spp</i>	-	1 (1.3%)
<i>Aspergillus oryzae</i>	1 (0.6%)	-
Total	167 (68.7%)	76 (31.3%)

Table 1: Total number of fungal isolates from rice and cowpea.

Fungal isolates	White Exposed	Brown Exposed	White Un-exposed	Brown Unex-posed	Total
<i>Rhizopus spp</i>	12 (75%)	9 (45%)	10 (55.6%)	9 (40.9%)	40 (52.6%)
<i>Aspergillus niger</i>	1 (6.25%)	3 (15%)	1 (5.6%)	7 (31.8%)	12 (15.8%)
<i>Aspergillus flavus</i>	1 (6.25%)	4 (20%)	2 (11.1%)	1 (4.5%)	8 (10.5%)
<i>Syncephalas-trum spp</i>	-	-	2 (11.1%)	2 (9.1%)	8 (10.5%)
<i>T. mentagro-phytes</i>	1 (6.25%)	-	2 (11.1%)	-	3 (3.9%)
<i>M. canis</i>	-	-	-	2 (9.1%)	2 (2.6%)
<i>Aspergillus fumigatus</i>	-	-	1 (5.6%)	1 (4.5%)	2 (2.6%)
<i>Scopulariop-sis spp</i>	1 (6.25%)	-	-	-	1 (1.3%)
Total	16 (21.1%)	20 (26.3%)	18 (23.7%)	22 (28.9%)	76 (100%)

Table 2: Distribution of fungal isolates according to different varieties of exposed and unexposed cowpeas.

Figure 2: Distribution of fungal isolates according to different varieties of exposed and unexposed rice.

Key: E1 U1= Abakaliki; E2 U2= Adani; E3 U3 = Oduma;
E4 U4= Ugbawka and E5 U5 = Foreign.

Discussion

Rhizopus species were the highest fungi isolated in the varieties of cowpeas and rice studied. A total of 40 (52.6%); 48 (28.7%) *Rhizopus* species out of 76 (31.3%) cowpeas and 167 (68.7%) rice fungal isolates encountered respectively. There was a statistical significance difference between the two cereals worked upon ($P < 0.05$). The reason could be due to the fact that *Rhizopus* species are thermotolerant saprobes and so the heat generated upon storage favour their growth [22]. The second predominant fungi encountered were *Aspergillus niger* and *Aspergillus flavus* 12 (15.8%); 8 (10.5%) and 44 (26.3%); 18 (10.8%) in both cowpeas and rice respectively. This could be attributed to their wide distribution in nature, being saprophytes in the soil, decaying vegetation and other substances according to Madunagu and Umana [23]; Onyeka and Ugwu [24]. This also agrees with the work of Somorin and Bankole [25], who reported that *Aspergillus* species were the most predominant fungi in “Ofada” and “Abakaliki” rice in Lagos

<i>Aspergillus spp</i>	No of isolates	White Exposed	White Unexposed	Brown Exposed	Brown Unexposed
<i>Aspergillus fumigatus</i>	2 (9.09%)	0	1	0	1
<i>Aspergillus niger</i>	12 (54.5%)	1	1	3	7
<i>Aspergillus flavus</i>	8 (36.4%)	1	2	4	1
Total	22	2 (9.1%)	4 (18.2%)	7 (31.8%)	9 (40.1%)

Table 3: Distribution of *Aspergillus* species according to different varieties of cowpeas.

<i>Aspergillus spp</i>	No of isolates	Exposed	Unexposed	Frequency (%)
<i>Aspergillus niger</i>	44	20	24	52.4
<i>Aspergillus flavus</i>	18	9	4	21.4
<i>Aspergillus fumigatus</i>	13	2	1	15.5
<i>Aspergillus tamarri</i>	3	1	1	3.6
<i>Aspergillus clavatus</i>	2	1	1	2.4
<i>Aspergillus restrictus</i>	2	1	1	2.4
<i>Aspergillus caesiellus</i>	1	1	0	1.2
<i>Aspergillus oryzae</i>	1	0	1	1.2
Total	84	43	41	100

Table 4: Distribution of *Aspergillus* species according to different varieties of rice.

Fungal genera	Abakaliki	Adani	Oduma	Ugbawka	Foreign	Total
<i>Aspergillus spp</i>	24 (28.6%)	12 (14.3%)	13 (15.5%)	11 (13.1%)	24 (28.6%)	84 (50.3%)
<i>Rhizopus spp</i>	11 (23%)	9 (18.8%)	8 (16.7%)	8 (16.7%)	12 (25%)	48 (28.7%)
<i>Trichophyton spp</i>	3 (18.8%)	2 (12.5%)	2 (12.5%)	8 (50%)	1 (6.3%)	16 (9.6%)
<i>Mucor spp</i>	3 (33.3%)	1 (11.1%)	-	-	5 (55.6%)	9 (5.4%)
<i>Geotrichum spp</i>	-	-	4 (50%)	4 (50%)	-	8 (4.8%)
<i>Syncephalastrum spp</i>	-	-	1 (50%)	1 (50%)	-	2 (1.2%)
Total	41 (24.6%)	24 (14.4%)	28 (16.8%)	32 (19.2%)	42 (25.1%)	167

Table 5: Distribution of fungal isolates according to genera in different varieties of rice.

Fungal genera	White	Brown	Total
<i>Rhizopus spp</i>	44 (31.4%)	96 (68.6%)	140 (66%)
<i>Aspergillus spp</i>	12 (27.3%)	32 (72.7%)	44 (20.8%)
<i>Trichophyton spp</i>	6 (33.3%)	12 (66.7%)	18 (8.5%)
<i>Syncephalastrum spp</i>	4 (50%)	4 (50%)	8 (3.8%)
<i>Scopulariopsis spp</i>	2 (100%)	-	2 (0.9%)
Total	68 (32.1%)	144 (67.9%)	212

Table 6: Distribution of fungal isolates according to Genera in different varieties of cowpeas.

and Ogun states of Nigeria. Hence there was a statistical significant difference between the *Aspergillus* species isolated from cowpeas and rice varieties in this work ($P < 0.05$). These results were shown in table 1. Among the cowpeas varieties it was observed that unexposed brown cowpeas had the highest number of fungal isolates 22 (28.9%) with *Rhizopus* spp ranking highest 9 (40.9%). Mycelia sterilia were seen among the *Scopulariopsis* spp in the brown cowpea exposed and unexposed. Also, the same were recorded among the *Syncephalastrum* spp of white and brown cowpeas exposed varieties. Some dermatophytes like *Microsporon* and *Trichophyton* spp recorded maybe as a result of presence of superficial mycoses from unhygienic practices accompanying the retailers according to Gallardo, *et al.* [26] and Kayser, *et al* [27]. Most probable reason could be due geophilic nature of these dermatophytes. These results were shown in table 2. There was no statistically significant difference between the fungal isolates of the exposed white and brown cowpeas and the unexposed white and brown cowpeas in this work ($P > 0.05$). In table 3, there was a tremendous increase in the total number of fungal isolates in the unexposed foreign rice variety followed by Abakaliki rice. These were represented graphically in figure 1. This agreed with the work of Somorin and Bankole [25] as earlier mentioned. Among the genera isolates *Aspergillus* species ranked highest 84 (50.3%) followed by *Rhizopus* species 48 (28.7%) out of 167 genera fungal isolates in rice varieties while *Rhizopus* species 140 (66%) ranked highest followed by *Aspergillus* species 44 (20.8%) in the cowpeas varieties. The reason could be that, according to retailers due to the chemicals they normally spray on these cereals during storage do not stick on the rice and swifts off easily on the cowpeas soft seed coat after some time attracting weevils and some rodents which deposit their excretas on them. Under high humidity and temperature favour the growth of moulds. This agrees with the work of Dawar, *et al.* [28] who found out that *Aspergillus* species are saprophytic and will continue to grow on pulses as long as conditions of high temperature and relative humidity support it. Hence, there was a statistically significant difference between the number of *Aspergillus* species in rice and cowpeas ($P < 0.05$). These are shown in table 5 and 6 respectively.

Conclusion

This study has demonstrated that cowpeas both exposed and unexposed harbour fungal agents and have the ability to transfer these pathogens to humans, causing fungal infections such as aspergilloma. This study demonstrates that the so called “foreign”

rice has no advantage, in terms of safety, over the local rice. Rice grains exposed and unexposed of both local and “foreign” accommodate the growth of fungal agents and have the ability to cause diseases in humans animals alike.

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