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INCIDENCE OF MOULDS IN SOME VARIETIES OF STORED COWPEA AND PEANUTS IN MAIDUGURI NIGERIA

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ABSTRACT

Incidence and types of mould present in three varieties of peanuts (Ex-Poland, Double colour and Yar Dakar) and cowpea (D.90, GV and Borno Brown) stored in Bulumkutu market, Maiduguri were studied. Moisture content, percentage seeds surface-infected, total mould count (surface and inner infection) and mould identification were determined. Moisture content ranged from 13.5 - 19.6% and 15.0 - 20.0% in varieties of peanuts and cowpea respectively. Varieties of stored cowpea had a wider range of surface infection (19.0-51.0%) compared with peanuts (21.3%-38.4%). Total mould count was however lower in cowpea (9.2×10^2 - 1.8×10^3 cfu/g) compared with peanuts (1.3×10^3 - 1.8×10^3 cfu/g), indicating that peanuts had more mould growing within the seeds than cowpea. Three genera of mould were identified in each crop - *Aspergillus. Penicillium* and *Zygomycete.* Borno Brown variety of cowpea had the highest load of these mould (57.6%) while GV had the least (14.3%). In peanut, Ex-Poland variety had the highest load of mould (42.9%) while each of Double colour and Yar Dakar variety had 28.6% load.

Key Words: Cowpea, peanuts stored, mould, incidence, Nigeria

INTRODUCTION

Cowpea *[Vigna unguiculata (L.)* Walp.] and peanuts *(Arachis hypogaea L.)* provide substantial part of the protein intake in the diet of many people in the developing countries, where starchy root crops and/or cereals form the basis of the diets (Akinnusi, 1986). The amount of cowpea and peanuts produced in Nigeria as a whole is sizeable. Production figures in 2002 were 477.8 kg/ha and 1070.1 kg/ha for cowpea and peanuts respectively (CAB International, 2003).

Several workers have studied the effect of moulds on agricultural commodities (Ayrest, 1969; Moline and Kuti, 1984; Chupp and Sherry, 1992). In Nigeria, such works include that of Ogbonna and Pugh (1983), Popoola *et al.* (1997), Popoola and Idakwo (2002). In all these, the researchers drew attention to the existence of these moulds and the effects they have on food commodities.

Akinnusi (1986) observed that fungal infestation of agricultural commodities was becoming alarming. This was attributed partly to lack of good packaging materials for transporting foodstuffs from the farms and partly to the storage facilities that had provided environmental conditions conducive for effective growth of moulds.

These moulds largely produce mycotoxins, the potency of which varies from one geographical location to another (Austwick *et al.,* 1960; Semnick *et al.,* 1970; Moubasher and 'Abdel-Hafez, 1978). It is therefore important for local researchers to document the species of fungi found in foodstuff in their own areas and highlight the potentials of various mycotoxicoses existing in the area (Bullerman, 1979).

The study area in this work is Maiduguri, a town in the north-east region of Nigeria and is situated within longitude 13° 00' -13° 45'E and latitude 11° 30'- 12° 00'N. The town is a part of vast expanse of land south of Sahara popularly referred to as semi-arid land. This paper reports the results of an aspect of a systematic research into level of mould contamination of local feed and foodstuff in Maiduguri, Nigeria.

MATERIALS AND METHODS

Collection of samples of cowpea and peanuts

Samples of cowpea *[Vigna unguiculata (L.)* Walp.subsp. *unguiculata]* and peanuts *(Arachis hypogaea L.)* were obtained from Bulumkutu market, Maiduguri. The peanuts varieties were Ex-Poland, Double colour, and Yar Dakar; while that of cowpea were D.90, GV(Kananado Type), and Borno Brown. The crop varieties were identified by the officials of Borno State Agricultural Development Programme. The legumes were part of a bulk harvested between the months of November 1998 and January 1999. They had been stored for about 3-4 months and later retailed in Bulumkutu market. They were procured for the work in mid-April, 1999.

Determination of moisture content

Moisture content was determined by drying 10 grams of ground samples in an oven set at 60°C for peanuts and 105°C for cowpea (Mossel, 1975). Measurements were taken daily until a constant weight was obtained for each crop. Percentage moisture content was then determined, taking average of three replicates.

Surface sterilization of cowpea and peanuts

Fifty seeds of each variety were soaked in 10% Milton® (antiseptic solution) for 2 minutes, with occasional stirring using a pair of forceps. The antiseptic solution was discarded and the seeds rinsed in two changes of sterile distilled water.

Isolation of moulds from infected cowpea and peanut seeds

Two culture media - Potato Dextrose Agar (PDA) and Yeast Extract Agar (YEA) were prepared and kept molten at 40°C. Chloramphenicol (100 ppm) and Rose Bengal (150 ppm) were then added to inhibit the growth of bacteria and restrict the growth of Mucorales respectively. The culture media were then poured into plates and allowed to set.

Ten surface-sterilized seeds of each variety were transferred onto the PDA plates using a pair of disinfected forceps. Sufficient gaps were left between the seeds to avoid spurious results due to cross contamination. The plates were incubated for 3 days at 30°C. Three replicates were set up. Seeds with fungal growth on them were counted and results expressed as percentage seeds invaded by mould.

Total mould count

Ten surface-sterilized seeds of each variety were blended using sterile blender. One gram of ground sample was diluted to 10^2 factor and 1 ml ox it plated on PDA plates in triplicates (Onion *et al.,* 1980). The plates were incubated at 30°C for three days. The emerging fungal colonies were counted using a colony counter. Results were corrected for dilution and expressed as colony-forming units (cfu) per gram sample.

Sub-culturing and identification of moulds present

Mould colonies appearing on plates for enumeration were sub-cultured to give pure colonies employing method of Onion *et al.* (1980). Culture slides, prepared for identification purposes, were mounted on microscope and examined for characteristic morphological features of vegetative and reproductive components. The moulds were identified following the guidelines recorded by Suyarnamanan (1970) and Onions *et al.* (1980).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) while the means were separated using Fischer's LSD (P=0.05).

RESULTS AND DISCUSSION

The moisture content of varieties of stored cowpea ranged between 15.0±0.6% and 20.0±0.2%, while that of peanuts varieties ranged between 13.5±1.5% and 19.6±0.6% (Table 1). The values were higher than those of other workers. Barampama and Simard (1993) recorded moisture content of 9.2% for some varieties of dry beans. Mills and Woods (1994) observed a production of off-flavour in peanuts having moisture content of 14.5%. The high moisture content recorded in this work might be connected with the storage conditions of most stores in Maiduguri. Past work (Popoola *et al.,* 1997) had shown high relative humidity (cir. 76%) in most stores few months before commencement of rain, especially when the heat was at the peak. Of importance also was the moisture level of the seeds at the time of intake for storage. If they were not properly dried, the chances of appreciable drying while in store would be very low. Moreover dry produce could easily reabsorb moisture from the atmosphere, unless stored in sealed containers. Jute bags were commonly used here and could hardly prevent reabsorption of moisture from the store micro-environment.

Table I Moisture content of varieties of cowpea and peanuts

Varieties	*% Moisture*
A: Cowpea	
1. D.90	*15.0+0.6a*
2. GV (Kananado Type)	*16.0±1.0a*
3. Borno Brown	*20.0+0.2b*
Mean	*17.0+2.1*
SED(±)	*0.5*
B: Peanuts	
1. Ex-Poland	*19.6±0.6b*
2. Double Colour	*14.8±0.3a*
3. Far Dakar	*13.5±1.5a*
Mean	*16.0+2.6*
SED(±)	*0.7*

Values are means of 3 replicates.
Means with same letters under a crop are not significantly different(p>0.05) according to F-LSD

The high moisture content of the stored seeds had a number of implications on their intended use. Nahdy *et al.* (1999) observed that high moisture content in pigeonpea actually led to increased predominance of active (flight) morphs of bruchids - *Callosobruchus chinensis (L).* The high moisture content in cowpea and peanuts stored in Maiduguri can therefore render the seeds more susceptible to fungi and insect infestation. High moisture content had also been linked with predisposition to fungal attack (Mills and Woods, 1994) and higher rate of sprouting in the seeds (Ismail *et al.,* 1997).

The results of mould infestation of cowpea and peanuts varieties are shown in Table 2. The figures represented surface infestation rather than the fungi growing within the **grains.** A combination of surface and internal infestation was represented by total mould count (Table 3).

Table 2. Percentage seeds invaded by mould

Varieties	! % seeds invaded
A: Cowpea	
1. D.90	31.2±2.Oa
2 GV (Kananado Type)	19.0±4.0b
3 Borno Brown	51.0+9.0c
Mean	33.7+13.2
SED(±)	4.7
B: Peanuts	
1. Ex-Poland	38.4±3.0a
2. Double Colour	21.3±2.2b
3. Yar Dakar	28.0±3.5c
Mean	29.2±7.0
SED(±)	2.3

values are means of 3 replicates.
Means with same letters under a crop are not significantly different(p>0.05) according to F-LSD

The greatest incidence of surface contamination (51.0±9.0%) was found in 'Borno Brown' variety of cowpea. There was, on the average, a higher percentage incidence of seeds invaded in cowpea than peanuts (Table 2). However total mould counts were higher in peanuts (1.5 x 10^3 cfu/g) than cowpea (1.2 x 10^3 cfu/g). This implied that peanuts had more internal infestation than cowpea. This might be due to higher moisture content in peanuts. Moreover, peanuts seemed to be natural habitat for most *Aspergillus* species. Mills and Woods (1994) observed that peanuts had higher levels of *Aspergillus glaucus* than cowpea even at the same level of moisture content.

Table 3. Total mould count (cfu/g sample) of crops

Varieties	Total mould count (x 10' Cfu/g)
A: Coupe D.90	9.2±3.0a
2 GV (Kananado Type	{ 10.0+2.0a
Borno Brown	18.0+5.0b
Mean	12.4±4.0
SED(±)	2.9
B: Peanuts	
Ex-Poland	l 3.5±3.0a
2. Double Colour	18.0±6.0b
3. Yar Dakar	12.5±3.5a
Mean	14.7±2.4
SED(±)	3.6

values are means of 3 replicates.
Means with same letters under a crop are not significantly different(p>0.05) according to F-LSD

Table 5 gives the frequency of occurrence of fungal species in the varieties of cowpea. The most frequently occurring fungal species was *Rhizopus stolonifer,* which occurred on all the varieties. All the four isolated fungi occurred on 'Borno Brown'. This variety incidentally had the highest moisture content (Table 1).

In peanuts, *Mucor pusillus* was the most predominant fungal species (Table 5). This fungus is a very ubiquitous thermophilic species found on most substrates all over the world (Eicker, 1972; Ogbonna and Pugh, 1983). It was sometimes associated with unsaturated fatty acids and could then be found on substrates containing these substances. It has also been known as the causal agent o l' a lung mycosis and other internal infections in various animals (Davis *et al.,* 1975).

D'Mello and Macdonald (1997) noted the presence of certain species of *Mucorales* and *Ascomycetes* in stored forage and gave

Table 4. Species of fungi present in varieties of stored cowpea

Fungal Species	D.90*	GV	Borno Brown °
1.Aspergillus ochraceus	+		+
2.Penicillium digitatum	-		+
3.Penicillium lividium			+
4.Rhizopus stolonifer	+		+
Total presence			
Percentage presence	28.6	14.3	57.6

* (+) = present; (-) = absence

Table 5. species of fungi present in varieties of stored peanuts

Fungal Species	Ex-Poland*	Double colour	Yar Dakar
l .Aspergillus ochraceus			+
2.Penicillium notatum	+		
3.Penicillium spinudosum	-	+	
4.Mucor pusillus	+	+	+
Total presence	3	2	2
Percentage presence	42.9	_ 28.6	28.6

* (+) = presence; (-) = absence

this as the major cause of mycosis of many ruminants. Also, *Mucor* species are saprophytic and produced an array of enzymes such as proteolytic and amylolytic group of enzymes (Popoola and Ogbonna, 1997). These enzymes break down the substrates on which the fungus grows and substantial part of the substrate's nutrients is used up by the fungi.

Aspergillus ochraceus was also common to the two crops. This species is well known for its production of ochratoxin (Hesseltine, 1979). Occasional isolations of the fungus from keratomycoses have been reported (Bullerman, 1979). The presence of these fungi in the seeds of the two crops calls for caution in their usage.

CONCLUSION

The stored cowpea and peanuts in Maiduguri, Nigeria have a load of mould in various proportions. 'GV' variety of cowpea was the least contaminated, while 'Borno Brown' variety was the most contaminated. In peanuts, 'Ex-Poland' variety was the most contaminated. Visual observation does not always reveal the presence of these moulds. It is therefore important that buyers and

consumers bear

this in mind as they put the commodities to various uses, either as feed or foodstuff.

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