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**RESEARCH ARTICLE**

# MYCOFLORA OF STORED PARKIA BIGLOBOSA (JACQ.) R.BR. EX G.DON (LOCUST BEAN) SEEDS FROM MARKETS IN JOS, NIGERIA AND CHANGES IN THEIR NUTRITIONAL COMPOSITION

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**ABSTRACT**

Fungal deterioration of stored *Parkiabiglobosa (*locust beans*)* seeds was investigated in four markets in Jos North, Plateau State, Nigeria. The incidence of fungi on locust bean seeds from the different markets were as follows: Katako (90%), Angwan Rukuba (76.7%), Gada Biu (86.7%) and Farin Gada (70%) respectively for the deteriorated seeds and Katako (10%), Angwan Rukuba (23.3%), Gada Biu (13.3%) and Farin Gada (30%) for the non-deteriorated seeds. There was a significant difference (P≤0.05) in the occurrence of fungi from the different markets for both deteriorating/non-deteriorating seeds; Katako (153/25), Angwan Rukuba (99/14), Gada Biu (109/22) and Farin Gada (36/14). A total of seventeen fungal species were isolated with *Aspergillus* species being the most dominant. The analysis of nutritional qualities of non-deteriorated and deteriorated locust bean seeds shows that the deteriorated seeds had reduced protein (9.07%), fibre (3.62%) and carbohydrate (23.37%) contents than the non-deteriorated ones. The study demonstrates that fungi is capable of causing damage to stored *Parkia biglobosa* seeds and contribute to low nutritional worth of the seeds. The health implications of the effects of these spoilage fungi were discussed.



## INTRODUCTION

The seed of Locust bean (*Parkia biglobosa*) plant found growing in Savannah area of Africa provides one of the popular seasonings in African diet. This nutritious and delicious food spice is popularly called “ogiri” in Igbo, “iru” in Yoruba or “dadawa” in Hausa in Nigeria. It is used to enhance food flavour and nutritional values. It is heavily consumed in Nigeria, Ghana, Sierre Leone and Togo (Odunfa, 1985). It serves as a readily source of protein for most of the people whose protein intake is low due to high costs of animal protein sources.

The plant occurs in a wide range of Natural Savannah woodlands and has the capacity to withstand drought conditions because of its deep tap root system. It is important in a wide variety of ways which include food, gum or resin from its mucilage, fodder from the pods for domestic animals, fibre and fuel as firewood and also in medicinal practices in the treatment of such ailments as bronchitis, pneumonia, malaria, diarrhoea and as a poison antidote. The roots are used as a lotion for sore eyes (Farombi, 2003). Although micro-organisms of all groups including bacteria, protozoa, algae, viruses, fungi, together with insects and rodents play significant roles in food deterioration; the most active and more versatile organisms that affect locust bean seeds and its products causing spoilage when stored are several species of bacteria and fungi (Omafuvbe *et al*., 2000). They can occur on growing crops as well as harvested commodities leading to damage ranging from rancidity, odour and flavor changes and germ layer destruction (Cutler, 1991).

In a study to identify the bacterial and fungal flora of deteriorated and maggot infested ‘Iru’ samples (fermented locust bean seeds) and determine the in vitro antibiotic susceptibility/resistance patterns of the isolated bacterial species using antibiotic discs and drugs assays. The isolated fungal species were identified as *Aspergillus niger*, *Aspergillusflavus*, *Penicillium*, *Rhizopus*, and *Candida* species. *Parkiabi globosa* (Locust bean) seeds are subject to degradation induced by diverse organisms including fungi which are among the most active micro-organisms in these processes (Popoola and Akueshi, 1985).

Micro-organisms associated with fermented locust bean seeds have been widely studied (Odunfa, 1981; Ikenebomeh *et al*., 1986; Odunfa and Oyewole, 1986; Ogbaduand Okagbue, 1988). Bacilli and Staphylococci were observed to dominate the fermentation together with a number of fungi. There is a dearth of information on fungal species causing deterioration of these seeds especially in storage in Northern Nigeria. The current study was aimed at isolating and identifying the fungal

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species associated with the deterioration of *Parkiabi globosa* and the proximate analysis of both decayed and healthy locust bean seeds in Jos, Plateau State, Nigeria.

## MATERIALS AND METHODS

The survey was carried out in four popular markets in Jos North Government Area of Plateau State, Nigeria which included Katako, Angwan Rukuba, Gada Biuand and Farin Gada markets. The seeds both deteriorated and non-deteriorated were collected from the markets in sterilized, well labeled plastic containers and were transported to the laboratory for processing. The healthy seeds of *Parkiabi globosa* are shown in Figure 1, while the deteriorated seeds from the different markets are shown in Figure 2.

### *Isolation and identification of fungal isolates*

The Locust bean seed samples were analyzed using the modified standard blotter method of (Anon, 1976).The decayed locust bean seeds were surface sterilized with 1% Sodium hypochloride and then rinsed in three changes of sterile distilled water. Ten seeds were placed in a sterilized Petri plate containing three well moistened blotting papers. Another set of experiment was set up with seed samples that were not deteriorated. Each of the treatments was replicated three times. The Petri plates were incubated at 27±2$^{o}$C 15 days. The blotting papers were moistened on a regular basis with little quantity of sterile distilled water to avoid dryness. The plates were observed daily for the emergence of colonies of fungi and the species were then transferred to Petri dishes containing already prepared Potato Dextrose Agar medium supplemented with gentamycin (40mg/ml). Growths observed were sub-cultured severally to obtain pure cultures of the fungal isolates. The pure fungal isolates were identified using their colony characteristics and microscopic morphologies. References were made to existing identification manuals (Raper and Fennel, 1965; Samson *et al*., 1984; Pitt, 1985; Onion *et al*., 1981; Nagamani *et al*., 2006).

### *Determination of moisture content*

The method of Ogbonna and Pugh (1982) was used for the assessment. For the moisture content determination, a weight of 30 grams of soil from each soil sample was dried to a constant weight in hot air oven set at 110$^{o}$C. The percentage moisture context was calculated by computing the loss in weight on drying as a fraction of initial weight of samples and multiplied by 100 and then recorded. This experiment was done in triplicates.

### *Proximate Analysis of Parkiabi globosa Seeds (Infected and Uninfected)*

The proximate compositions of the infected and uninfected samples were determined using standard procedures of AOAC (1995). The parameters determined were protein, ash, crude fibre, fat and carbohydrate. The crude protein content was calculated by multiplying the total nitrogen with the factor 5.4, using Kjedahl method, Bello *et al*. (2008) and crude fibre by AOAC (1995). The amount of lipid (fat) was determined using Soxhlet extraction method; while the ash content was determined by the method of AOAC (1995), and the carbohydrate content of each sample was determined by difference.

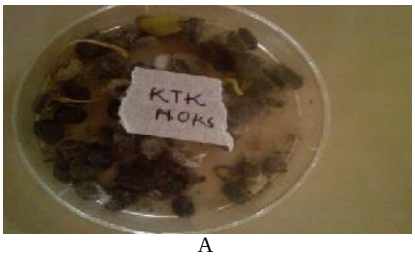
### *Statistical analysis*

Analysis of variance was used to analyse the data obtained and one-tailed student's t-test was used to test for level of significance (P≤0.05).

## RESULTS AND DISCUSSION

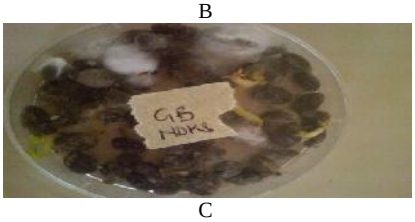
A total number of seventeen fungal species belonging to nine genera were isolated from the study. The different genera

**Figure 1** Seeds of Parkiabiglobosa (Locust Beans)




A

B



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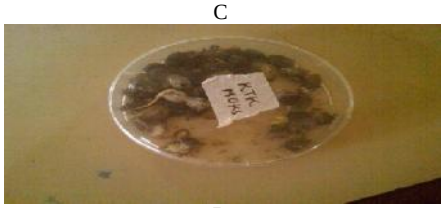

D

**Figure 2** Deteriorated locust bean seeds from the different markets- (a) Katako (b) Angwan Rukuba(c) GadaBiu (d) FarinGada

included *Aspergillus* with five species (29.41%),*Chaetomium, Cladosporium*, *Fusarium* and *Penicillium* (11.76%) had two species respectively. The remaining genera, *Absidia, Curvularia, Mucor* and *Rhizopus* (5.88%) had one species each. *Aspergill usniger* was the most dominant species, followed by *Fusariumo xysporum*, then by *F. solani* and finally by *A. flavus*. Figure 3a-3d showed the frequency of the isolated fungi from the four markets under study. The deteriorating samples harboured the majority of the fungal isolates from the four markets sampled. Katako market had the highest number (153/25) of fungal isolations both from the deteriorating / non- deteriorating samples, it was followed by GadaBiu market (109/22), Angwan Rukuba market (99/14) and finally FarinGada market (36/14). Some of the fungal isolates are presented in Figures 4a-4c.

It is evident that all the isolated fungi were found to be commonly associated with all the locust bean seeds from the four markets except for *Aspergill us can did us* which was not isolated at all from Angwan Rukuba market. *Absidia corymbifera, Cladosporium herbarium* and *Mucorh aemalis* were also not isolated at all from Farin Gada market. Most of the fungal isolates including genera of *Aspergillus, Chaetomium, Cladosporium, Mucor, Penicillium* and *Rhizopus* have been implicated in post-harvest rot of some fruits and vegetables in storage (Adaskaveg, 2002; Fagbohun and Lawal, 2011). *Curvularia* and *Fusarium* species are known pathogenic fungi Anwar *et al* (1996) and their isolation from the deteriorating samples indicates that they were seed-borne and must have followed the seeds right from the field. Their presence in the sampled seeds can cause up to 30% reduction in yield of the locust bean seeds. Isolation of these fungal species has health implications as some of them produce mycotoxins, carcinogenic agents (Campbell-Platte, 1980) which are elaborated into the food substance. These mycotoxins are heat stable, and are dangerous th the system when ingested even in very minute concentrations. *Aspergillus* species especially *A. niger* have been reported as opportunistic pathogens, causing asthma and otomycosis in compromised individuals(Austwick, 1965).Korzeniowska, (1990) reported a pulmonary aspergilloma caused by *A. niger*.

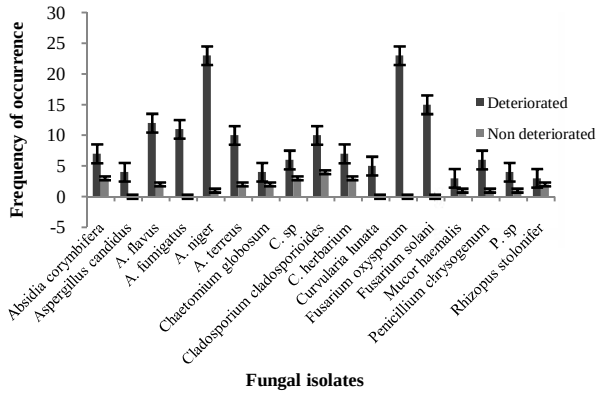


**Figure 3a** Frequency of occurrence of fungal isolates from Katako market

*A. niger* and *Mucor* sp are worldwide in distribution and have been isolated from various habitats including stored foodstuffs. *Aspergillus* has been reported as one of the predominantly isolated microbial species in Nigeria (Ogaraku, 2010). The author also reported that the fungi *Aspergillus* and *Penicillium* cause discolouration, and mouldy smell of locust bean seeds during storage. Sharma and Vir (1986) reported *A. niger* as the cause of rotting in numerous food products including fruits and vegetables, thereby causing huge economic losses due to spoilage.

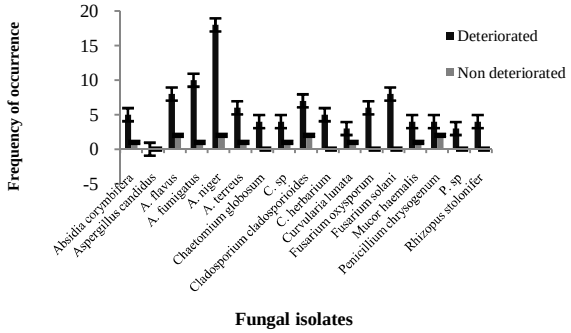


**Figure 3b** Frequency of occurrence of fungal isolates from AngwanRukuba market

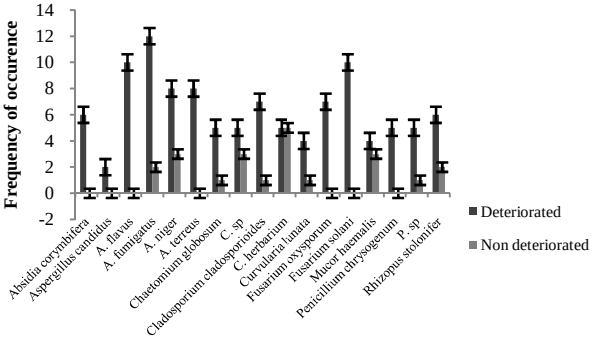


**Figure 3c** Frequency of occurrence of fungal isolates from GadaBiu market

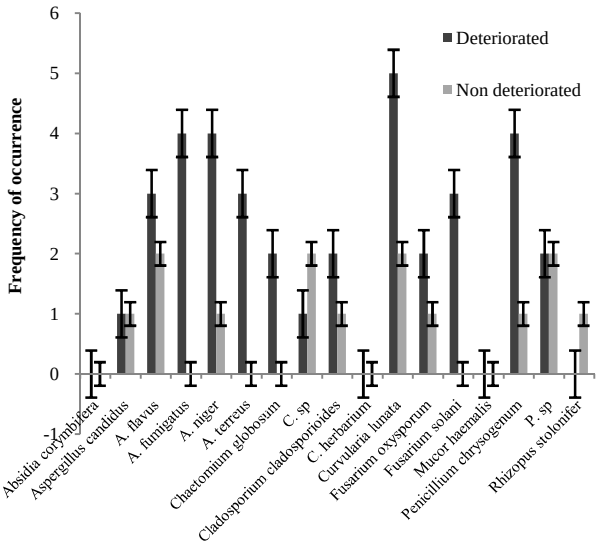


**Figure 3d** Frequency of occurrence of fungal isolates from FarinGada market

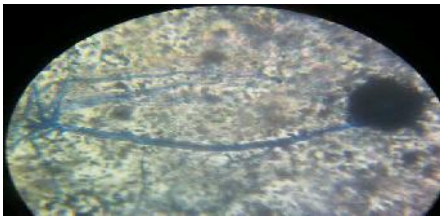

**Figure 4a** *Aspergillus niger*

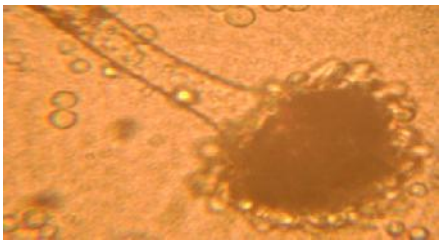

**Figure 4b** *A. flavus*

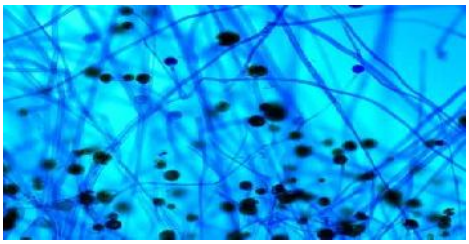

**Figure 4c** *Muco rhaemalis*

The incidence of fungi on locust bean seeds from different markets in Jos North is shown in Table 1. It was observed that out of the 30 seeds sampled from each market, the deteriorated samples had more incidences of fungi (90%, 76.7%, 86.7% and 70%) in comparison to the non-deteriorated samples (10%, 23.3%, 13.3%, 30%). This result is in line with that of Javaid *et al*. (2005) who reported high incidence of seed-borne fungi in black chickpea. They also reported that due to the high fungal infestation, the seeds of this species exhibited poor germination percentage of below 50%.

**Table 1**Incidence of fungi on locust bean seeds from different markets in Jos North Local Government Area

| Market | Total No. of samples | Deteriorated | Non-Deteriorated |
|---|---|---|---|
| Katako | 30 | 27 (90) | 3 (10) |
| AngwanRukuba | 30 | 23 (76.7) | 7 (23.3) |
| GadaBiu | 30 | 26 (86.7) | 4 (13.3) |
| Faringada | 30 | 21 (70) | 9 (30) |
| **Total** | **120** | **97 (80.8)** | **23 (19.2)** |

**Figures in parenthesis are percentages**

The analysis of proximate composition carried out on the locust bean seeds both deteriorated and non-deteriorated are presented in Table 2. The values for moisture (6.35%), fat (30.5%) and ash (3.88%) contents in the non-deteriorated seeds were significantly lower (P≤0.05) than in the deteriorated seeds; with moisture (10.75%), crude fibre (5.26%), fat (45.5%) and ash (6.10%). The values of protein (9.59%), crude fibre (5.26%) and carbohydrate (46.06%) in the non-deteriorated seeds were higher than in the deteriorated seeds with protein (9.07%), crude fibre (3.62%) and carbohydrate (23.37%). The decrease in the protein content of the non-deteriorated and deteriorated seeds was not significantly different (P≤0.05).

**Table 2** Proximate Composition of non-deteriorated and deteriorated Locust Bean seeds

| Seed Condition | Moisture Content | Crude Protein | Crude Fiber | Fat | Ash | Carbohydrate |
|---|---|---|---|---|---|---|
| Non-Deteriorated (%) | 6.35 | 9.59 | 5.26 | 30.50 | 3.88 | 46.06 |
| Deteriorated (%) | 10.75 | 9.07 | 3.62 | 45.5 | 6.10 | 23.37 |

The present findings have showed that the proximate composition of deteriorated locust bean seeds had decreased compared with healthy or non-deteriorated ones. This showed that the colonisation of the seeds by the storage fungi reduced significantly the nutrient composition of the seeds. This reduction in the nutrient values of the locust bean seeds is an evidence of utilisation of the nutrient contents of the seeds by the fungi.

In this study, moisture content increased from 6.35% in Non - deteriorated seeds to 10.75% in the deteriorated bean seeds. The higher content of moisture in the deteriorated seeds could be attributed to maceration of cellulose and pectin components of the beans cell wall by the biodeteriogens. It could also be as result of the biodeterioration activities of the spoilage fungi with the resultant elaboration of their metabolites into the surrounding bean seeds and the dissolution of the beans tissues into rot thereby increasing the moisture level of the bean seeds. This result coincides with that of Falaye and Fagbohun(2012) who also reported an increase in moisture content from 5.09% in the non-infected *Arachis hypogea* seeds to 6.13% in the infected ones. Ataga and Umechuruba(1997) also reported an increase in moisture content of African yam bean inoculated with storage fungi.

Similar increase was observed in the fat (45.5%) and ash (6.10%) contents of the deteriorated bean seeds to that of the non-deteriorated seeds with fat (30.50%) and ash (3.88%). The increase in the fat content of the deteriorated locust bean seeds could be as a result of the fact that the fungal species could not metabolize the fat in the bean seeds; instead they released some

of the fats globules that are accumulated in their bodies into the seeds. However, fat and ash are high energy nutrients just like carbohydrate, and does not add to the bulk of the diet as reported by Atasie *et al*. (2009). Similar result was obtained by Sanyaolu *et al*. (2010) who had earlier observed an increase in fat content in diseased *Irvingiagabonensis*.

It was revealed that the crude protein (9.59%), crude fibre (5.26%) and carbohydrate (46.06%) contents of the
Non-deteriorated seeds were higher than in deteriorated seeds with protein (9.07%), crude fibre (3.62%) and carbohydrate (23.37%). This is in agreement with the reports of Shehu and Aliero, (2010) who reported that infected onion leaf had a significant decrease in the crude protein and crude fibre contents. The reduction in the protein content of the seeds could be as a result of the biodeterioration activities of the fungal isolates which must have acted on those nutrients, hydrolyzing them for their survival. This is in line with the results of Nweke and Ibiam (2012) in their work on *Annona muricata*. It is important to note that apart from the effect of deterioration in the nutritional content of food crops as a result of activities of fungi, the mycotoxins produced by this group of microorganisms are of serious public health importance (Sanyaolu *et al*. 2014). Therefore, there is need to educate the public on the need to use fungus free locust bean seeds for dietary purposes.

The reduction in the crude fibre implies that locust bean seeds diet will be deficient in fibre and this could lead to diseases of the colon such as cancer and other intestinal disorders including constipation.

In conclusion, the seeds of locust bean plant are very important source of nutrients especially proteins that could alleviate malnutrition among different sections of Nigerian population and beyond. The seed is a readily source of protein for most of the people whose protein intake is low due to high costs of animal protein sources. The study has shown that the colonization of locust bean seeds by storage fungi had a significant impact in reducing the nutritional worth of the seeds. This situation can be improved by taking some measures including; drying of the seeds properly to reduce the moisture content which encourages microbial growth and spoilage. Oerke and Dehne (2004) reported that the present of little moisture permit the growth of fungi such as *Aspergillus* and *Pencillium* species.

Secondly, Farmers, traders and consumers should maintain sanity in the storage facilities which will go a long way to prolong the shelf life of the seeds. Considering the previous works on fungal deterioration of agricultural produce including locust bean seeds, the farm products are generally susceptible to fungal attack mostly because of the relative humidity and moisture which is always high in the storage areas.

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