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# EFFECTS OF STORAGE METHODS AND MYCOFLORA ON PROXIMATE COMPOSITION OF AFRICAN YAM BEAN (*Sphenostylis stenocarpa* Hochust ex Rich ) SEEDS

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## ABSTRACT

One of the limitations of African yam bean (AYB) (*Sphenostylis sternocarpa*) is poor storage ability due to the adverse effect of seed-borne fungi. This study was conducted to examine the effects of storage methods on nutritive composition of AYB seeds stored in three types of storage materials viz; jute bags, polypropylene bags and plastic bowls. Freshly harvested AYB seeds were stored in all the storage materials for 6 months using 2 × 3 factorial (2 AYB cultivars and 3 storage methods) in 3 replicates. The proximate analysis of the stored AYB seeds was carried out at 3 and 6 months after storage using standard methods. The temperature and relative humidity of the store room were recorded monthly. Seeds stored in jute bags gave the best values for crude protein (24.87%), ash (5.69%) and fat content (6.64%) but recorded least values for crude fibre (2.55%), carbohydrate (50.86%) and moisture content (12.68%) at the 6th month of storage. Temperature of the store room decreased from 32.9 ºC - 28.3ºC, while the relative humidity increased from 78% - 86%. Decreased incidence of field fungi namely: *Rhizopus oryzae*, *Aspergillus flavus, Geotricum candidum, Aspergillus fumigatus* and *Mucor meihei* was accompanied by increase in storage fungi viz: *Apergillus niger, Mucor hiemalis, Penicillium espansum* and *Penicillium atrovenetum* with prolonged storage. The study showed that out of the three storage materials, jute bag was more effective in preserving AYB seeds.

**Keywords**: African Yam Beans, Mycoflora, Storage Materials, Storage Methods and Preservation.

## INTRODUCTION

African yam bean (*Sphenostylis stenocarpa*), is an underutilized tropical African tuberous legume. There are seven species in the genus *Sphenostylis* (Potter and Doyle, 1994). African yam bean (AYB) is the most valuable. The arable tuberous legume is important in most indigenous African food cultures and in peasant agriculture. AYB is important economically and grown mainly in the Southern parts of Nigeria for its edible seeds (Potter, 1992). Although, it is generally considered a minor crop and under-exploited (Nwokolo, 1987; Saka *et al*., 2004), usually cultivated in association with yam, cassava, maize, sorghum and other crops (Togun and Egunjobi, 1997). The plant produces underground tubers that are used as food in some parts of Africa, and grown for household consumption and rarely for commercial purposes in Nigeria (Saka *et al*., 2004).This indigenous crop has potential for food security in Africa. AYB seeds are well balanced in essential amino acids and have higher amino acids content than what is found in pigeon pea, cowpea, and bambara groundnut (Uguru and Madukaife, 2001). Cooked seeds of African Yam Bean have higher fibre content, high efficiency of protein digestibility, higher amino acid availability, high gross and metabolizable energy and good fatty acid profile (Nwokolo, 1996). Despite its great potential to meet adequate nutritional requirements, it has been referred to as an under exploited, under-utilized and a neglected crop (Jaenicke and Pasieeznik, 2009). The cultivation of *S. stenocarpa* is still at the subsistence level, because its potential as an important crop that could meet the nutritional needs of Africans have not been explored.. Seeds when improperly stored deteriorate and spoil during preservation for two reasons either as a result of insufficient drying of the seeds before storage or as result of the presence of small quantities of spores of storage fungi which may be present on seeds going in to storage (VanEgmond *et al*., 2007). Storage fungi are adapted to grow in an environment of low moisture content and high osmotic pressure for deterioration of stored seeds (Alexopoulos *et al*., 2007). Seeds of crops can also loose values in store due to harsh and inappropriate storage conditions. Storage medium is one of the factors that influence preservation of seeds in storage. This study investigated the effect of storage methods on nutritive composition of two different cultivars of African yam bean seeds.

## MATERIALS AND METHODS

### *Sample collection*

Two different cultivars (Owo white and Owo brown) of freshly harvested *S. stenocarpa* seeds were obtained from Owo, Ondo State Nigeria.

### *Storage of seeds*

One kilogramme each of freshly harvested seeds of the two cultivars were separately packaged in the three storage materials: jute bag, polypropylene bag and white plastic bowl having the following sizes respectively 9.31m$^2$, 9.31m$^2$ and 40.19m$^3$. The assessment was carried out on the samples monthly. The experimental design used for the seeds storage was 2 x 3 factorial design (involving two cultivars × three types of storage containers) in a completely randomized design. The mouths of the storage containers were tied tightly with pieces of string and the plastic bowl covered with lid. The bags and bowls were placed on raised platforms to prevent moisture absorption from the concrete flooring in the room. The temperature and relative humidity of the store room were regularly recorded with kestrel pocket weather tracker 4000.

Thirty grammes (30g) sub sample each of the stored seeds were collected from the storage materials on monthly basis for six months. At each sampling, isolation of moulds from the stored seeds subsamples was done. The proximate analysis was carried out on subsamples at the third and sixth month of storage.

### *Isolation and identification of moulds from stored AYB seeds*

The direct seed plating techniques on agar media was employed for the isolation of fungi from the AYB seeds (ISTA, 1976). For the direct plating on agar media, the Potato Dextrose Agar (PDA) was prepared according to the manufacturer's description with the addition of 0.1% streptomycin. After the prepared agar had solidified, 10 seeds of African Yam Bean seeds subsamples collected on a monthly basis were aseptically plated on PDA (10 seeds per plate) and were incubated for 5 – 7 days at 28±2°C. Representative colonies of fungi that appeared on agar plates were aseptically sub cultured on fresh PDA until pure culture of each isolates were established. The colonies of each pure culture agar plates were later transferred to PDA slants for further use.

The morphological features of fungi isolates were identified under the compound light microscope using cultural method according to the method described by Barnett and Hunter, (1998) and compared with a pictorial compendium (Tsuneo, 2002).

***Proximate Analysis:***
The proximate analysis was carried out using the standards of AOAC (1998).The following were determined: moisture content, fat content, ash content, crude fiber content, crude protein content and carbohydrate content.

## RESULTS AND DISCUSSION

The moulds isolated from the African yam bean seeds were basically grouped into two categories namely field fungi: *Rhizopus oryzae*, *Aspergillus flavus*, *Geotricum candidum, A. fumigatus, Mucor meihei* and storage fungi viz: *Apergillus niger, M. hiemalis, Penicillium espansum, P. atrovenetum* (Figure 3-6). This result conforms with the report of Fagbohun *et al*. (2011) who stated that *Aspergillus niger, Aspergillus candidus, Aspergillus flavus, Aspergillus glaucus, Aspergillus versicolor and Rhizopus sp*. were found to be associated with the stored sundried soyabean seeds, most of which are known to be surface contaminants of agricultural products. The findings from this study is in accordance with reports of researchers that *Rhizopus spp, Penicillium notatum, Aspergillus niger, A. flavus* and *Fusarium oxysporum* are found to attack seeds in field and storage (Fagbohun *et al*., 2010; Ghangaokar and Kshirsagar, 2013). The decreased incidence of field fungi during storage with time and by six month of storage conforms with the work of Bankole *et al*. (1999). In all the storage materials and cultivars, the populations of *R. oryzae, G. candidum, M. meihei, M. hiemalis, A. fumigatus* and *A. flavus* decreased significantly (ranging from 100% - 64.7% reduction) as shown in Figures 3-8. The decreased incidence of field fungi accompanied by increase of storage fungi could be as a result of availability of metabolized nutrient by other fungi present and the environmental conditions that influence the nature and density of the colonizers (Okigbo, 2003). Decrease in the presence of moulds in jute bags (Figure 3) during period of storage was probably due to low seed moisture content. Some researchers have reported that the seed mycoflora increases with the increase in seed moisture which results in seed rotting and loss of seed quality (Mazen *et al*., 1993; Paderes *et al*., 1997). This can also be explained by the porosity of jute bag which might serve as a medium of free exchange of moisture between the inside of the storage material and the surrounding environment. The variation in occurrence of *A. flavus* (Figure 4) could be attributed to the differences in prevailing weather conditions that occur at the time of storage. The conditions of the stored product determine the extent of invasion of the stored product. Figure 1 and 2 shows the mean temperature and relative humidity profiles of the store throughout the whole period of the study. The duration of seeds in storage is influenced by the stored seed quality as well as storage conditions. Irrespective of initial seed quality, unfavorable storage conditions, particularly temperature and relative humidity contribute to accelerating seed deterioration in storage. The environmental factors that aid the development of fungi in stored products include moisture content (Amusa *et al*., 2002), temperature (Abakar-Gyenin and Norman, 2000), aeration (Giampietro, 2004), pH (Aderiye, 2004) and relative humidity (Sinha *et al*., 2014). Decrease or increase in seed moisture in relation to relative humidity and temperature of storage air has been well documented (Bewley and black, 1985; Chrastil, 1990; Walters, 1998; Copeland and McDonald, 1999; McDonald, 1999; Rehman and Shah, 1999; Volenik *et al*., 2006). The decrease in AYB seed moisture content after 6 months of storage in all the storage materials should lead to prolonged seed viability since 1% decrease in seed moisture content doubles the viability of seeds (Copeland and McDonald, 1999). However, viability of seeds is not only influenced by relative humidity and consequently seed moisture content but also with seed temperature. Reduction in seed temperature doubles the viability of the seed (Copeland and McDonald, 1999; Harrington, 1973).The storage period had a significant effect on the proximate composition of African yam bean seeds. The effect of cultivar and storage materials on the proximate composition of African Yam Bean (AYB) at different storage period is shown on table 1. Irrespective of storage materials, the ash content, carbohydrate content and crude fibre of the brown AYB at 3 and 6 months after storage were significantly ($P<0.05$) higher than the content in white cultivar. The crude protein in the brown and white cultivar of AYB at 3 months after storage were 24.24 and 23.76 respectively but not significantly ($P>0.05$) different from each other. However, the crude protein content (23.50) in the brown cultivar at 6 months of storage was significantly ($P<0.05$) higher than the content (22.66) in white cultivar (Table 1). The Crude protein is significantly high at the third month after storage. This agrees with the work of Ameh (2007) who reported a high percentage of Crude protein on three accessions of African yam bean. However, there was slight reduction in the crude protein after six months of storage in all the storage materials with the least value observed in plastic bowl. This is in accordance with the observation made by Hellevong (2000) who reported that protein deteriorates with time as a result of microbial activities. This also conforms with Amakoromo *et al*. (2012) who reported that the crude protein content of AYB yoghurt from whole seeds decreased from 6.44 to 6.38%, during 4 days of storage at room temperature, while the sample from dehulled seeds decreased from 5.40 to 5.10%. Although, the two cultivars of African yam beans showed different crude protein values, the high biological value of the proteins require that greater attention should be given to this crop in the humid tropics where the crop is generally accepted, the result of the analysis showed that the Ash content, Carbohydrate content and Crude fibre contents of African yam bean seeds were lower than those reported for other legumes. It is clear from this research work that the seed of *S.stenocarpa* composed mainly of protein rather than carbohydrate, ash and crude fibre. Also storage materials exhibited significant effect on the proximate composition of AYB, the physical characteristics of jute bag allowed an exchange of air and moisture between the seeds and the environment than all other storage materials while the least interactions with the surrounding environment was ensured by the plastic bowls. However during the more humid months of the year which was observed as the storage period advanced, there was retention of moisture in the plastic bowl due to a reduced exchange of water vapour with the environment leading to higher moisture in seed stored in plastic bowls. The seed stored in jute bags which has the largest pores was found to have the least moisture content of all the storage materials. At the end of the storage period there was significant increase in the value of ash content in AYB seeds regardless of the storage materials used (Table 1). Similar results were observed by Radunz *et al*. (2004) with different storage methods in different periods concluded that regardless of the storage system, maize seeds showed significant increase in ash content, reflecting in a reduction of quality. There was reduction in fat content at the end of the storage period, regardless of storage materials used, with values ranging from 7.95- 6.64 (jute bag), 6.85- 5.58 (polypropylene bag) and 5.36- 4.42 (plastic bowl) as can be seen in table 2. The results of this study are similar to those obtained by Gutkoski *et al*. (2009), which showed a reduction in lipid content during the storage of dried corn seeds stored in fence granary with forced natural air. Likewise, Abreu *et al*. (2013), working with sunflower seed concluded that oil content in seeds declined over time regardless of storage condition. The increase in carbohydrate in this study can be related to the decrease between the protein and fat fractions during storage (Schuh *et al*., 2011). The presence of a higher carbohydrate in seeds stored in plastic bowls may have been occasioned by the inability of fungal cells to grow optimally in the vegetative forms which makes use of carbohydrate for metabolism. In jute and polypropylene bags that have pores which encourage low moisture, there is probability of a continuous growth of fungi which must have been able to use the organic carbon in form of carbohydrate. The nutritional benefits derivable from African yam bean seeds can be harnessed by reduction of anti-nutrient contents and formulation of infant foods and diet (Brough *et al*., 1993; Poulter and Caygill, 2006). The result of this study revealed that the longer the seeds of AYB are stored, the higher the rate of reduction in nutritive value of the seeds. Thus, the rate of deterioration of the seeds will be low in view of the little moisture level observed in the two cultivars.

**Table 1: Effects of cultivar and storage materials on proximate composition of AYB seeds**

| | Ash | | Carbohydrate | | Crude fiber | | Crude protein | | Fat | | Moisture | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Months** | **3** | **6** | **3** | **6** | **3** | **6** | **3** | **6** | **3** | **6** | **3** | **6** |
| **Cultivar** | | | | | | | | | | | | |
| Brown | 4.32a | 5.25a | 51.07a | 52.04a | 2.72a | 3.53a | 24.24a | 23.5a | 7.35a | 6.09a | 13.68a | 11.84a |
| White | 3.34b | 4.23b | 50.38b | 51.40b | 2.11b | 2.90b | 23.76a | 22.66b | 6.10b | 5.01b | 13.07a | 11.40a |
| **Storage Material** | | | | | | | | | | | | |
| Jute | 4.75a | 5.69a | 50.03c | 50.86c | 1.89c | 2.55c | 25.77a | 24.87a | 7.95a | 6.64a | 11.83c | 10.47c |
| Plastic bowl | 2.97c | 3.77c | 51.55a | 52.60a | 2.97a | 4.01a | 22.14c | 21.22c | 5.36c | 4.42c | 14.94a | 12.68a |
| Polypropylene | 3.78b | 4.77b | 50.60b | 51.71b | 2.38b | 3.09b | 24.10b | 23.16b | 6.85b | 5.58b | 13.37b | 11.71b |
| **Cultivar × Storage Material** | | | | | | | | | | | | |
| Brown × Jute | 5.23a | 6.11a | 50.39c | 51.21c | 2.15c | 2.87c | 25.89a | 25.09a | 8.40a | 7.00a | 11.81c | 10.25c |
| Brown × Plastic | 3.45c | 4.40c | 51.91a | 53.00a | 3.22a | 4.46a | 22.71c | 22.07c | 6.23b | 5.22c | 15.60a | 13.31a |
| Brown × Poly | 4.27b | 5.25b | 50.90b | 51.92b | 2.79b | 3.25b | 24.13b | 23.35b | 7.41a | 6.04b | 13.63b | 11.94b |
| White × Jute | 4.26a | 5.27a | 49.66c | 50.51c | 1.62c | 2.23c | 25.65a | 24.65a | 7.50a | 6.27a | 11.85c | 10.68c |
| White × Plastic | 2.48c | 3.14c | 51.19a | 52.20a | 2.72a | 3.55a | 21.56c | 20.36c | 4.50c | 3.63c | 14.27a | 12.05a |
| White × Poly | 3.28b | 4.30b | 50.29b | 51.49b | 1.98b | 2.93b | 24.07b | 22.97b | 6.29b | 5.12b | 13.11b | 11.48b |

Means along the same columns with different alphabets are significantly different ($p < 0.05$) using Duncan Multiple Range Test.

Foot note: AYB - African Yam Bean; Poly - polypropylene bag.

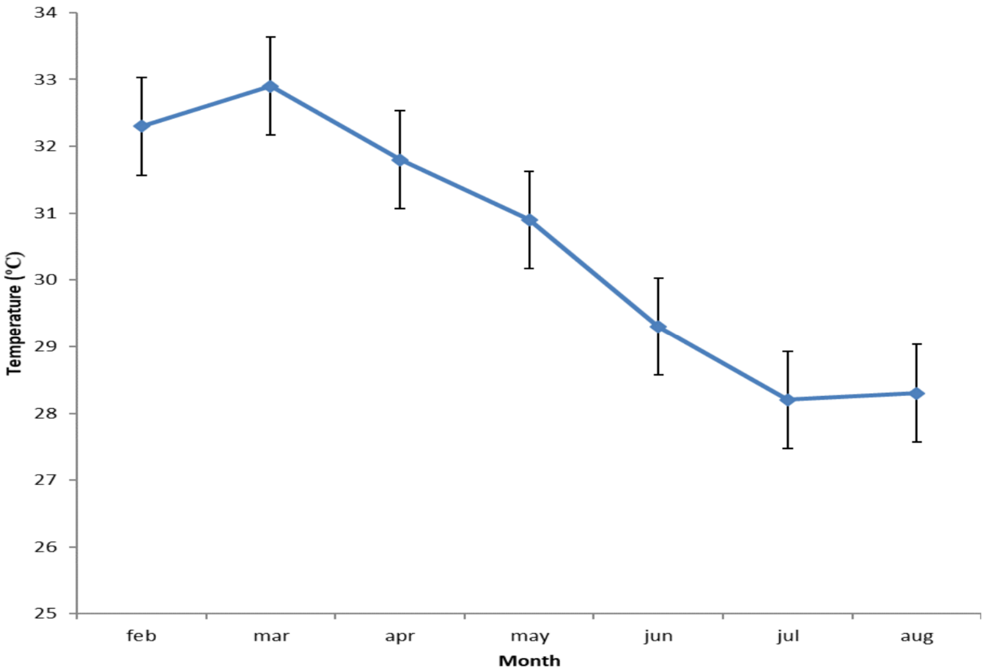


**Figure 1: Mean monthly Temperature profiles of the store over 6 months storage of AYB seeds cultivar white and brown in jute bags, polypropylene bags and plastic bowls.**

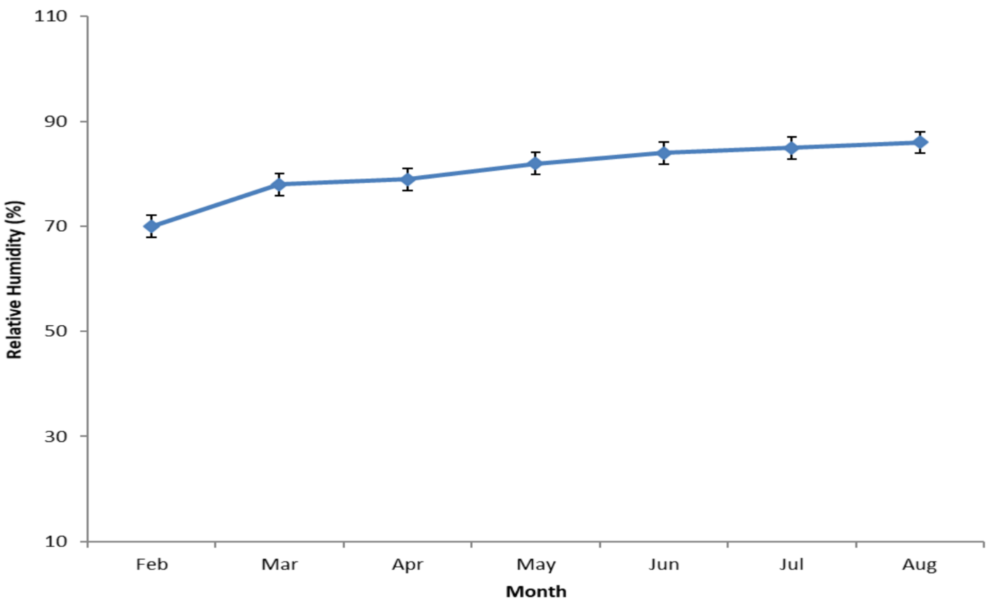


**Figure 2: Mean monthly Relative humidity profiles of the store over 6 months storage of AYB seeds cultivar white and brown in jute bags, polypropylene bags and plastic bowls.**

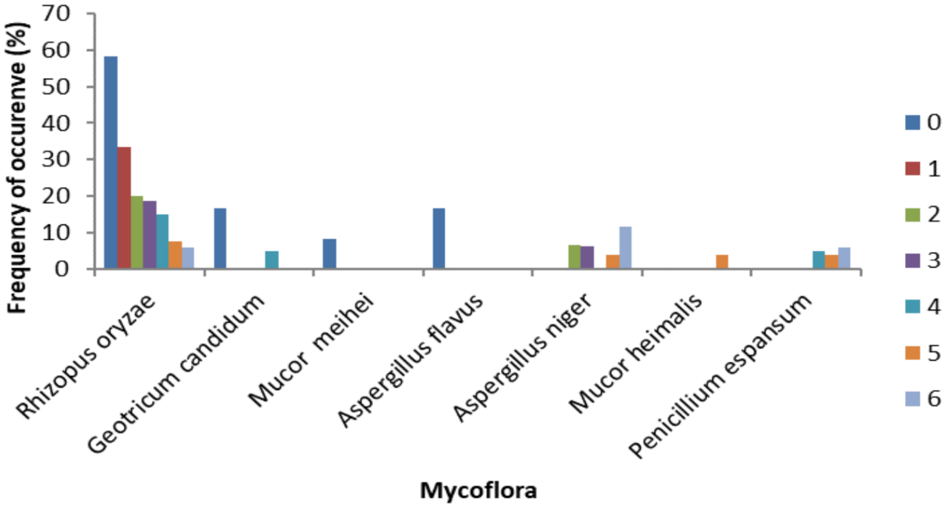


**Fig 3: Percentage frequency of occurrence of fungi isolated from brown cultivar of AYB seeds stored in jute bags at 0 – 6 months.**

Key 0= unstored, 1= first month of storage, 2= second month of storage, 3= third month of storage, 4= fourth month of storage, 5= fifth month of storage, 6= sixth month of storage.

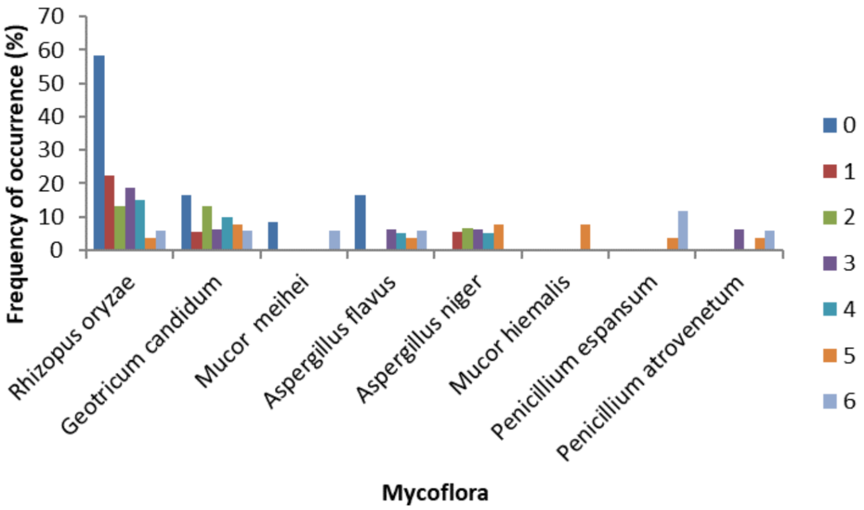


**Fig 4: Percentage frequency of occurrence of fungi isolated from brown selected cultivar of AYB seeds stored in polypropylene bags at 0 – 6 months.**

Key 0= unstored, 1= first month of storage, 2= second month of storage, 3= third month of storage, 4= fourth month of storage, 5= fifth month of storage, 6= sixth month of storage.

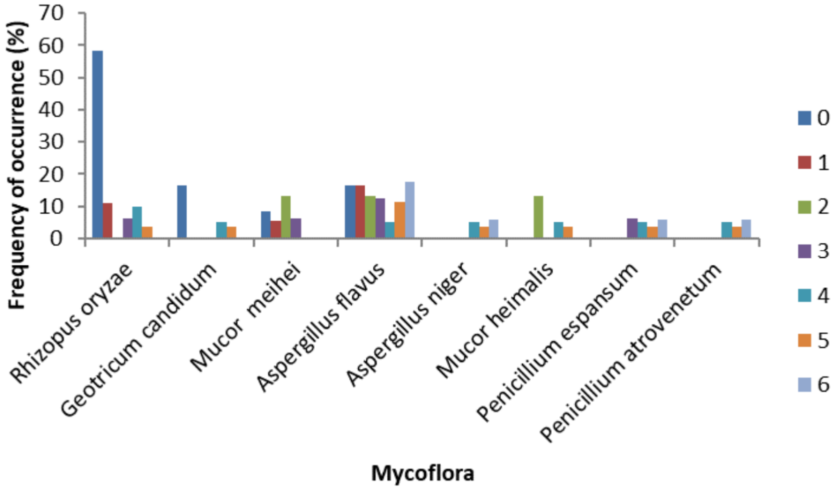


**Fig 5: Percentage frequency of occurrence of fungi isolated from brown cultivar of AYB seeds stored in plastic bowls at 0 – 6 months**

Key 0= unstored, 1= first month of storage, 2= second month of storage, 3= third month of storage, 4= fourth month of storage, 5= fifth month of storage, 6= sixth month of storage.

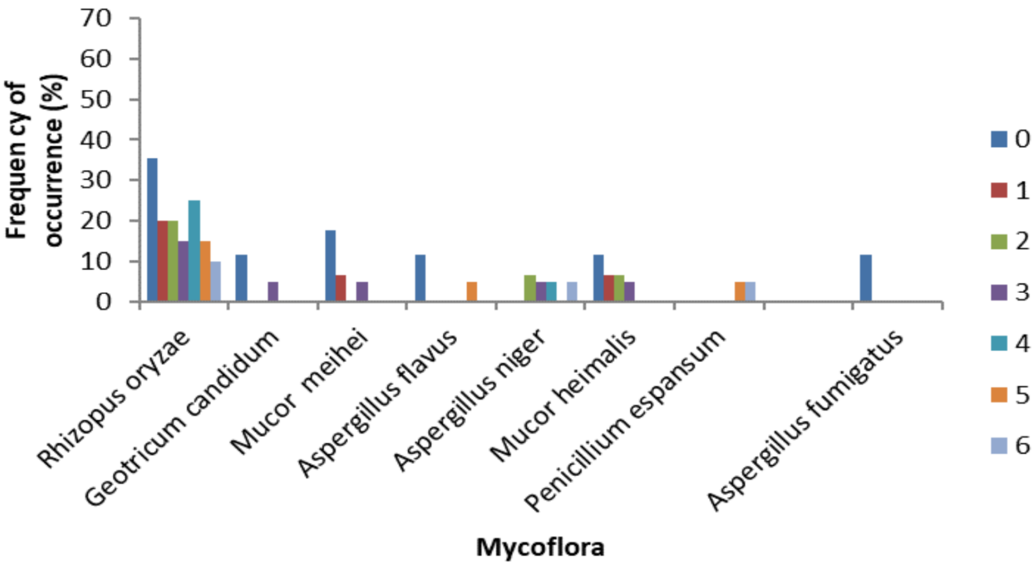


**Fig 6: Percentage frequency of occurrence of fungi isolated from white cultivar of AYB seeds stored in jute bags at 0 – 6 months**

Key 0= unstored, 1= first month of storage, 2= second month of storage, 3= third month of storage, 4= fourth month of storage, 5= fifth month of storage, 6= sixth month of storage.

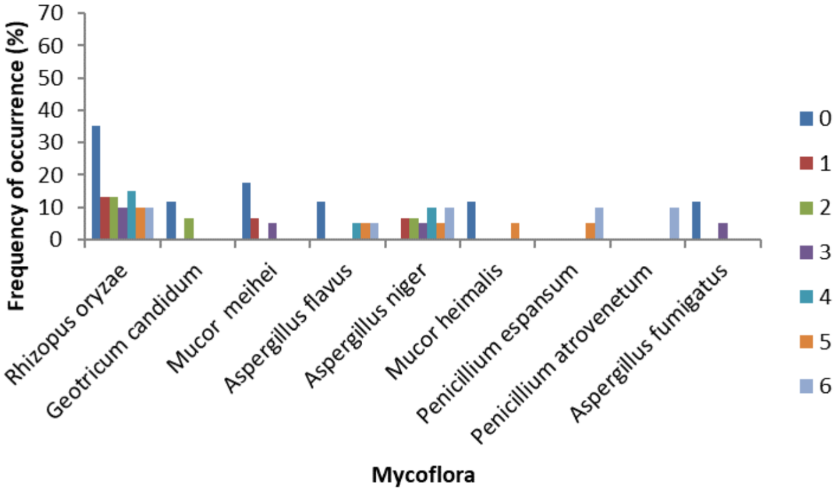


**Fig 7: Percentage frequency of occurrence of fungi isolated from white cultivar of AYB seeds stored in polypropylene bags at 0 – 6 months**

Key 0= unstored, 1= first month of storage, 2= second month of storage, 3= third month of storage, 4= fourth month of storage, 5= fifth month of storage, 6= sixth month of storage.

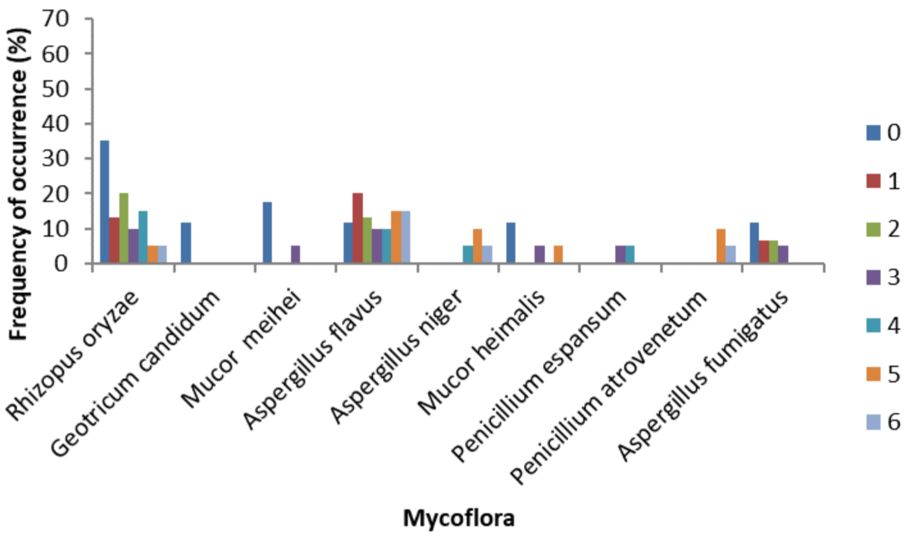


**Fig 8: Percentage frequency of occurrence of fungi isolated from white cultivar of AYB seeds stored in plastic bowl at 0 – 6 months**

Key 0= unstored, 1= first month of storage, 2= second month of storage, 3= third month of storage, 4= fourth month of storage, 5= fifth month of storage, 6= sixth month of storage.

## CONCLUSION

AYB seeds are of great economic importance. In order to maintain the quality, the seed should be stored under proper controlled environment that prevent them from fungal deterioration. The findings from this study indicate that jute bag was the most effective in preserving AYB for six months. Decreasing these losses due to microbial attack can be attained by providing suitable storage materials and proper post-harvest handling prior to storage. This will further improve the prospective use of AYB both locally and internationally to harness the potential of this great crop.

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