# Isolation and Identification of Fungi Associated with *Abelmoshus esculentus* (OKRA) Sold in Abuja, Nigeria

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**Abstract:** Isolation and identification of fungi associated with sun dried okra (*Abelmoshus esculentus*) sold in some selected parts of Abuja, Federal Capital Territory was investigated. Various fungi isolates were sub-cultured until pure cultures were obtained using pour plate technique. Determination of the pH and moisture content of the sun dried okra was carried out. The pH the samples ranged from 5.83 to 6.90, Kwali and Bwari council areas had the highest with 6.90, while Abaji council had the least with 5.83. Moisture content of Okra samples from Gwagwalada council was recorded as the highest with 18.62%, while samples from Abaji council had the least moisture content of 11.80. Fungal count across the council areas indicated Bwari had the highest with 2.0 x $10^5$CFU/mg, while the lowest fungi count occur in Kwali with 6.0 x $10^2$CFU/mg. The mould species identified in all the samples include; *Aspergillus flavus* 13(27%), *Aspergillus fumigatus* 9(18.4%), *Aspergillus niger* 6(12.5%), *Mucor* spp 4(8.3%), *Fusarium* spp 4(8.3%), *Aspergillus sydowii* 3(6.25%), *Aspergillus ustus* 3(6.25%), *Aspergillus candidus* 2(4.2%), *Penicillum* spp 2(4.2%), *Saccharomyces* spp 1(2.1%) and *Clasdoporum carrionii* 1(2.1%). Fungi isolated from Okra in this study could pose serious public health risk if consumed with time. Therefore, safety precautions should be employed in the processing and sun drying of Okra to avoid its contamination.

**Keywords:** Okra, pH, Moisture content, Fungi isolates.

## INTRODUCTION

Vegetables form a valuable part of human diet in some regions of the world; they contribute to the nutritive value of food in many developing countries. Vegetables are rich in minerals such as potassium, sodium, calcium, iron, zinc, and phosphorous (Ijeoma *et al.,* 2012).They play an important role in maintaining general health. Most vegetables are perishable due to high moisture content, so they are either consumed within few days or preserved for later consumption. Drying may be achieved by sun or using hot-air oven. Drying preserves food by reducing water activity of the food to a level insufficient for enzymes activity or the growth of microbes thereby preventing decay and spoilage (Ofor and Ibeawuchi, 2010). Drying enhances storage stability, reduces bulkiness of food, most perishable crops are dried so as to increase shelf-life and to promote food security. However, Okra is a perishable commodity which can deteriorate, and this can cause reduction in its quality and quantity if not properly handled during storage processes. According to De Lannoy (2001), despite the wide acceptability and consumption of this dried, powdered okra, little is known regarding the spoilage fungi, their toxigenic properties and nutritional contents. There is scarcity of fresh okra during the dry season as a result of poor storage and preservation methods. Okra (*Abelmoschus esculentus)* is an important vegetable crop grown in tropical, subtropical and worm temperate regions around the world. The edible part is the immature fruit; the fruit become fibrous and not suitable for consumption when fully matured. The most important okra producing countries include Indian, Nigeria, Pakistan, Ghana and Egypt. It is known as "Orun'la" in "Yoruba", "Kubewa" in "Hausa" and "Okwale" in "Igbo" languages of Nigeria (Tindall, 2010). Okra is a prominent fruit and leafy vegetable grown for domestic consumption of the highly nutritious immature leaves and fruits in Nigeria (Farinde *et al.,* 2007). Okra is a perishable commodity which deteriorates and this can cause reduction in its quality

and quantity during handling and storage processes. Traditionally, the fresh okra chips are indiscriminately sun-dried on roof tops, concrete constructions, and along roadsides for some weeks depending on the intensity of sunlight. This unhygienic act inevitably poses the risk of exposing the commodity to direct contamination or indirectly from dust, flies, rodents and even human handlers, which as a result, produce condition for fungal growth (Ofor and Ibeawuchi, 2010). Fungi are the major contaminants of foods in the world, and it has been reported that some fungi, mostly moulds can produce toxic secondary metabolites under unfavorable environmental conditions (Jonathan and Esho, 2010). Mycotoxins, for example, aflatoxin contamination of food have been reported and documented to be the cause of different types of abortion and cancer both in human beings and animals worldwide (Jonathan and Esho, 2010). Okra is usually available in large quantities during rainy season and scarce during dry season, there is the need to preserve them, and to give important considerations to this food safety and fugal contamination. Okra mucilage is suitable for medicinal and industrial applications, it has been medically found applicable in plasma replacement or blood volume expander, and it has also been reported in curing ulcers and relief from hemorrhoids (Siemonsma and Kauame, 2004). Industrially, the mucilage is used to glance setting papers and also useful in confectionery. Okra is one of the perishable commodities due to its high moisture content; the main traditional method of post-harvest preservation of the vegetable is by sun drying. The principal genera of field fungi are *Altenaria, Fusarium, Curvularia, Helmithosporium, Chaetomium,* and *Cladosporium*. All the field fungi require high seed moisture content for growth. The storage fungi consist of the genera *Penicillium, Aspergillus,* few species of yeast. Agricultural crops are usually infected by fungi both in field as well as stored ones. *Aspergillus*species, *Fusarium* species, *Pythium* species, *Phytophora* specie *and Penicilium* produce mycotoxin that causes food poisoning (Adebanjo and Bankole, 2003). Aflatoxins contamination diet is immunotoxic to both livestock and man. The level of mycotoxins and the occurrence of mould in food should be checked regularly and frequently monitored, because of their effects in humans and animals.

## MATERIALS AND METHODS

### Study Area Description

Abuja is the capital city of Nigeria. It's located in the center of Nigeria, within the Federal Capital Territory (FCT). Abuja is a planned city and built mainly in the 1980s. The City has a population of 776,298 as at 2006 National Population Census, with total land area of 8000km$^2$. Abuja lies 9$^0$03'60.00 North of the equator and 7$^0$28'59.99 East of the meridian. The council areas under study for this research are Kwali, Kuje, Gwagwalada, Abuja Municipal, Abaji and Bwari.

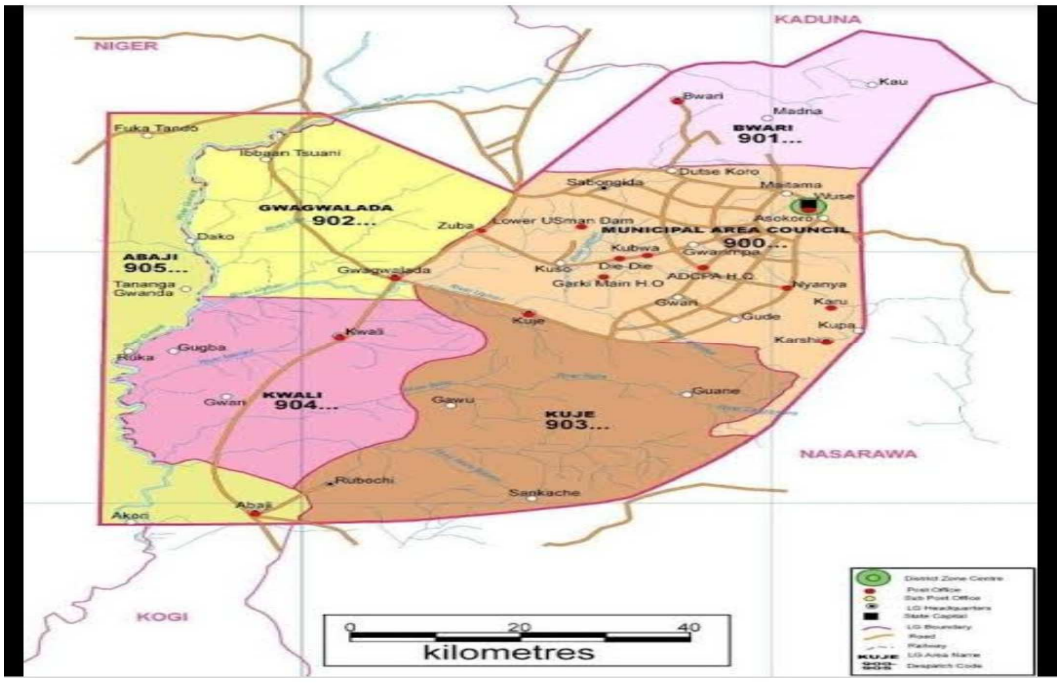


**Fig 1: Map of the Federal Capital Territory (FCT) showing the six (6) council areas where the samples were collected.**

**Collection of Samples**

Sun dried okra samples were purchased from six (6) different vendors in FCT council area markets. The samples were kept in sterile polythene bags and labeled according to the market and locations at the Biotechnology Laboratory of Sheda Science and Technological Complex (SHESTCO), and were transferred to the Department of Microbiology Laboratory, Ibrahim Badamasi Babangida University, Lapai, Niger State for further analysis.

**Preparation of the sample**

Exactly 10 g of the finely grinded powder of the sun-dried okra was dispensed separately into 100 ml of sterile distilled water. This was properly shaken to ensure homogeneity of the substance; after which it was kept in the refrigerator until needed.

**Determination of pH**

An EIL 7020 pH meter was used for the determination of the acidity of each of the samples; the pH meter was calibrated to a neutral pH, before it was used to determine the acidity level of the samples. Ten (10) g of the grinded powder of the sun-dried okra was dispensed separately into 100 ml of sterile distilled water; these were properly shaken. An electrode was then inserted into the mixture and stirred properly to ensure stable reading (Oyeleke and Gana, 2013).

**Determination of Moisture Content**

This is a measure of the % moisture lost due to drying at a temperature of 105$^{o}$C. Exactly 2g of the powdered okra was weighed ($W_1$) into pre-weighed crucible ($W_o$) and placed into a hot drying oven at 105$^{o}$C for 3hours. The crucible was removed, cooled in a desiccator and weighed. The process of drying, cooling and weighing were repeated until a constant weight ($W_2$) was obtained. The weight loss due to moisture was obtained by the equation:

$$\% \text{ Moisture} = \frac{W2 - W0}{W1 - W0} x\ 100\%$$

Where:

$W_o$= Weight of the empty crucible (g)

$W_1$= weight of the powder sample + empty crucible (g)

$W_2$= weight of dried sample + empty crucible (g) after oven drying (Josyln, 1970)

**Determination of Fungal count from Sun-dried Okra Using the Dilution Method (Pour-plate technique)**

Seventy (70) percent ethanol was used for the surface sterilization of the laminar flow hood. Ten (10) g of the sun dried okra was poured into 100ml of sterile distilled water, which was used as the stock. The solution was adequately shaken to make it homogenous and ensure that no clumping occurs. A pipette was used to aseptically dispense 1ml of the homogenized solution into a sterilized test tube, into which 9 ml of sterile distilled water had been dispensed. The whole set up was properly shaken and used for serial dilution up to $10^{-5}$. One (1) ml of aliquots of $10^{-2}$ and $10^{-4}$ were added to sterile Petri dishes, after which molten sterile Saboraud dextrose agar (SDA) was added to the Petri dishes. The Petri dishes were swirled gently to obtain a uniform mixture. Once solidified, the dishes were then incubated at room temperature for one week. However, during the incubation, the dishes were examined for fungal growth and colonies after every 48 hours (Ayalen *et al.,* 2006; Jonathan and Esho, 2010).

**Isolation and Identification of Fungi**

Successive hyphae tip was transferred to fresh media until pure cultures of each of the fungi were obtained. Pure isolate of the fungal culture was obtained by aseptically transferring fungal hyphae to freshly prepared SDA plates and incubated for seven days. This was done until pure cultures were obtained using single spore isolation technique. The pure isolates obtained were transferred to freshly prepared SDA slants for further studies. Each fungal colony was viewed under the microscope using x 40 magnification and then compared to Color Atlas of Mycology (Mutegi *et al.,* 2009; Negedu *et al.,* 2010; Adetunji *et al.,* 2012).

## RESULTS

The pH value and the moisture content of the sun dried okra samples obtained from six different retail outlet located in Abuja Area Councils market is presented in Table 1 below. The pH of the samples ranges from 5.83 to 6.90. Samples from Kwali and Bwari council areas had the highest pH value of 6.90, while the sample from Abaji council area had the least pH of 5.83. The moisture content percentage of the Okra samples ranges from 11.80 to 18.62. Samples from Gwagwalada had the highest moisture content of 18.62%, while samples from Abaji had moisture content of 11.80%.

**Table 1: pH and Moisture Content of Okra samples obtained from FCT**

| Location | pH | Moisture content (%) |
|---|---|---|
| Kwali | 6.90 | 15.25 |
| Kuje | 6.30 | 16.98 |
| Gwagwalada | 6.82 | 18.62 |
| AMAC | 5.90 | 15.22 |
| Abaji | 5.83 | 11.80 |
| Bwari | 6.90 | 14.64 |

The result obtained from the total fungal count of the samples from the different markets in Abuja, the Federal Capital Territory is presented in Table 2 below. This indicated that the highest total fungal count occurred in the sample obtained from Bwari area council market, with the value of 2.0 x $10^5$, while the lowest total fungal count occurred in the sample obtained from Kwali area council market, with the value of 6.0 x $10^2$.

**Table 2: Total Fungi count in Colony Forming Units from Okra samples obtained from FCT.**

| Location | Dilution factor | No of colonies | Fungal counts (cfu/mg) |
|---|---|---|---|
| Kwali | $10^{-2}$ | 6 | $6.0x10^2$ |
| | $10^{-4}$ | 4 | $4.0x10^4$ |
| Kuje | $10^{-2}$ | 10 | $1.0x10^3$ |
| | $10^{-4}$ | 7 | $7.0x10^4$ |
| Gwagwalada | $10^{-2}$ | 8 | $8.0x10^2$ |
| | $10^{-4}$ | 6 | $6.0x10^4$ |
| AMAC | $10^{-2}$ | 12 | $1.2x10^3$ |
| | $10^{-4}$ | 10 | $1.0x10^5$ |
| Abaji | $10^{-2}$ | 22 | $2.2x10^3$ |
| | $10^{-4}$ | 18 | $1.8x10^5$ |
| Bwari | $10^{-2}$ | 25 | $2.5x10^3$ |
| | $10^{-4}$ | 20 | $2.0x10^5$ |

The fungal colonies grew rapidly on the plate and produced different varieties of color such as green, yellowish green, white, purple, black, brownish black, ash, grayish green, yellowish brown and milky colonies. The fungi isolated were identified to specie level going by their physical and morphological appearances. The fungi were found to be: *Aspergillus niger, A. flavus, A. fumigatus, A. ustus, A.candidas, A. sydowii, Mucor* spp*, Fusarium* spp*, Clasdosporum carrionii, Yeast,* and *Penicillum* spp. The percentage distribution among the isolates indicated that *Aspergillus flavus* occurred most (27%), while *Saccharomyces* spp and *Cladosporumcarrionii* occurred least (2.1%) (Table3).

**Table 3: Distribution of Fungi species in Okra samples obtained from FCT**

| Fungi isolates | No. of isolates | % occurrence |
|---|---|---|
| *Aspergillus flavus* | 13 | 27.0 |
| *A. fumigatus* | 9 | 18.4 |
| *A. niger* | 6 | 12.5 |
| *Mucor* spp | 4 | 8.3 |
| *Fusarium* spp | 4 | 8.3 |
| *A sydowii* | 3 | 6.25 |
| *A ustus* | 3 | 6.25 |
| *A candidus* | 2 | 4.2 |
| *Pencillum* spp | 2 | 4.2 |
| *Saccharomyces* spp | 1 | 2.1 |
| *Clasdosporum carrionii* | 1 | 2.1 |
| Total | 48 | 100 |

## DISCUSSION

Moisture content is an important parameter in the storage of sun dried okra, very high levels of moisture content allows microbial growth and therefore, low levels are favoured for relatively longer shelf life. The highest moisture content of the sun dried okra sample was from Gwagwalada market (18.62%), while the lowest moisture content was the sample from Abaji market (11.80%), the moisture content decreased through drying due to evaporation of any possible water. The results of this study indicates that the variation in the moisture content maybe due to prolong storage in a cold environment, because these values were slightly higher than that of (Yousif, 1993) with average value of 6.13%. The variation in the moisture contents in all the market samples could be due to the temperature and relative humidity of their respective market locations, as well as the length of sun-drying. The pH obtained from the six markets had little variations which ranged from pH 5.83-6.90 and had values below the neutral pH of 7, this pH value indicates that dried okra sample are slightly acidic. Similarly, the report of Matazu and Suleiman (2002) and Kolawole *et al*. (2011) indicated that the internal pH of vegetables falls between the acidic range of pH 4.5 and 6.4.

Mould species isolated in this study are undesirable in food. *Aspergillus niger* was found to be present, it's one of the aflatoxin producing moulds whose presence in food would constitute health hazards to consumers. Highest fungal counts observed from the sun dried okra samples was from Bwari area council market ($2.0x10^5$)and the lowest fungal count was observed in Kwali area council market ( $6.0 x10^2$), the fungal counts could be attributed to higher moisture content, which makes Okra prone to easy microbial spoilage and subsequent short shelf life (Ijeoma *et al.,* 2012). *Aspergillus flavus* and *Aspergillus fumigates* had higher percentages of occurrence in all the samples, similar fungi isolates were reported on some selected Benin vegetables. Jonathan and Esho (2010) reported a similar fungus isolates on stored *Pluerotuso streatus and P. pulmonarius* (edible Nigeria Mushroom). The different observation from this study may be due to the different substrate used in respect to their moisture contents. It was also observed that the findings of this study is in agreement with that of (Farinde *et al.,* 2007), who isolated other related fungi as a bio-deteriorating organisms from street sold foods. Extra care must be taken while handling, harvesting, packaging, during storage and transporting of sun dried okra, and this is in line with the report of Ayalen *et al*. (2006). *Fusarium* spp were also isolated from this research and they are recognized for the production of lethal toxins known as Fumonisms. In the markets of Abuja, Federal Capital Territory, the extensive increasing occurrence of these fungi has severe implications, as some of them are recognized to produce mycotoxins. Fungi contamination of farm products is caused by the way the farm products, as well as food crops are handled during post-harvest transportation from the farm to the market places and homes which could lead to increase in fungi density in such products.

## CONCLUSION

The pH of the Okra samples recorded in the study was within the acidic range which might have favored the mould growth. The moisture contents of the samples were moderately high. The mould count observed also reflected the different moulds isolated across the sample areas in this study.

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