Influence of Using Different Drying Technique on the Level of Toxigenic *Aspergillus flavus* in Maize (*Zea mays*) Seeds

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**Authors’ contributions**

This work was carried out in collaboration between both authors. Author JAYA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JME managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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**ABSTRACT**

Deterioration of maize is mainly affected by moisture content, temperature, relative humidity, storage conditions period of drying and method, fungal growth, and insect pests. The growth of *Aspergillus flavus* in maize is facilitated by hot and humid conditions and they pose a major risk through production of mycotoxins. To maintain high-quality maize for both short- and long-term storage, maize must be protected from weather, growth of microorganisms, and pests. The Influence of drying on the toxigenic *Aspergillus flavus* level of maize seeds was investigated. Different drying method used are; constructed wooden solar dryer lined with aluminum foil, constructed wooden solar dryer lined with black polythene, calabash (*Crescentia cujete*), tray lined with plantain (*Musa paradisiaca*) leaf, tray lined with *Thaumatococcus daniellii* leaf. ] Maize seeds were inoculated with spore suspension of toxigenic *Aspergillus flavus*. The inoculated maize seeds were dried using different drying methods provided and the growth performance of the *A. flavus* was

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monitored on inoculating chamber with the spores counted at weekly interval. After three days of drying, inoculated maize grains dried with wooden solar dryer lined with aluminum foil and calabash were found to have the lowest spore levels of *Aspergillus flavus* counted with haemocytometer counting chamber (5.50x10^5 sfu/l and 9.50 x10^2 sfu/ml) as compared to the value of spores counted from the other drying method used. There was a continuous decrease in the values of moisture content in all the samples from day 1 to day 3. The highest reduction was also observed in samples of solar dryer lined with aluminum foil. Considering the different materials used for drying maize in this study, Calabash and solar dryer lined with aluminum foil proved to be more reliable and efficient in reducing the sporulation level of *A. flavus*

**Keywords:** Maize; drying method; toxigenic *Aspergillus flavus*; solar dryer; crop protection.

1. INTRODUCTION

Maize is a monocotyledonous and cereal plant of the species *Zea mays* and it is grown because of the grain it produces [1]. Maize is part of the world’s essential food grains such as rice, barley, wheat, oat and sorghum. It is a basic staple food grain for large parts of world including Africa, Latin America, and Asia [2]. The production of maize in the world has been increasing continuously [3]. Maize is a major important cereal being cultivated in the rainforest and the derived Savannah zones of Nigeria. Maize has been in the diet of Nigerians for centuries. It started as a subsistence crop and has gradually become more important crop. Maize has now risen to a commercial crop on which many agro-based industries depend on as raw materials. [4]. It can be processed into different food, beverages and feed ingredients serving as a source of food to both human and livestock.

Maize grains are subject to infection by a variety of toxigenic fungi, most commonly *Aspergillus spp., Fusarium spp., Alternaria spp.,and Penicillium spp.* [5-7]. Toxigenic *Alternaria* and *Fusarium* species are often classified as field fungi, while *Aspergillus* and *Penicillium* species are considered as storage fungi [8].

Storage is an important aspect of food security in developing countries. This is especially important since most cereals including maize, are produced on a seasonal basis and in many places there is only one harvest a year, which itself may be subject to failure. Seasonal production leads to fluctuating supply at the international, regional, national or at household levels. The fluctuating supply is in sharp contrast to a stable demand throughout the year and region. Storage helps to even out fluctuations in market supply, both from one season to the next and from one year to the next, by taking produce off the market in surplus seasons, and releasing it back onto the market in lean seasons [9].

Storage and drying have effect on the chemical, physical and biological properties of maize. The maize weevil and the larger grain borer (LGB) are the main and most serious pests of stored maize [10]. The LGB is a fairly recent pest, and its introduction has caused a wave of new research. It was accidentally introduced into Africa in the late 1970s from its area of origin in Central America, where it had long been recognized as an occasional pest of stored maize. It appeared first in East and then West Africa and is now widely recognized as the most destructive pest of farm-stored maize in Africa [11]. It can be now be found in many places in many countries in Africa.

Pre and post-harvest bio-deterioration and spoilage of grains, vegetables, fruits and agricultural produce due to infestation by pests, insects and microorganisms may cause losses of up to 100%. Fungal contamination of stored commodities is a very serious problem in tropical warm regions of the world [12]. Contamination with fungi diminishes the quality of grain because toxigenic fungi species produces a highly toxic compound known as mycotoxin. Fungal growth and toxin production in maize have been found to depend on several interacting factors which stress maize plants. Stress factors include low moisture content of soil, high daytime maximum temperatures, high nighttime minimum temperatures, and nutrient-deficient soils ([13].

Mycotoxins are the secondary metabolites of molds and invariably contaminate food and feed all over the world. Mycotoxin is the general name given to all toxin produced by the fungi species. Among hundreds of known mycotoxins, aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FBs), zearalenone (ZEA), and trichothecenes stand out as the most common contaminants in a
variety of food. Ingestion of these mycotoxins may cause acute toxicity or chronic disorders, depending on concentration and duration of exposure. Moreover, they are often responsible for financial losses in food production and livestock breeding [14].

Mycotoxins cause a whole range of disorders in the body of human and animal, ranging from biochemical changes through the functional and morphological damages of different tissues and organs, to the appearance of clinical signs of mycotoxicoses with even possible fatal consequences [15].

Contamination of stored product by storage fungi and their mycotoxins is of great concern in food industry. Fungi, especially the species of Aspergillus and Penicillium are among the major reported genera having the ability to produce mycotoxins during storage [16-17].

Fungi including species of Aspergillus are significant destroyer of foodstuff and grains during harvesting, storage, rendering them unfit for human consumption by retarding their seed quality and nutritive value and often by producing mycotoxins [18].

A significant portion of the agricultural products in the country and the world over become unfit for human consumption due to mycotoxins contamination of grains, especially those produced by species of Aspergillus [19].

Aspergillus species are known to produce a broad spectrum of mycotoxins including aflatoxins, sterigmatocystin and ochratoxins, which are causative agents of several carcinogenic, hepatogenic, nephrogenic, and immunosuppressive effects [20-23].

The main toxic effects are carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immunosuppression [24]. A sizeable portion of the world population living below poverty line in the developing and underdeveloped countries of Asia and Africa are suffering from health problems associated with consuming mycotoxin contaminated grains and cereals [25]. Even though effective and efficient control of seed borne fungi of seeds can be achieved by the use of synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity [26].

Thus, there is a need to search for alternative approaches to dry and store grains or cereals for human consumption without toxicity problems that are eco-friendly and not capital intensive.

The presence of Mycotoxin in some corn is unavoidable because contamination of maize by Aspergillus strains can occur during pre-harvesting, growth (while the seeds are on the plant), after harvesting, processing (when seed are dried) and during storage. In addition, the different biotic factors like temperature, moisture, and relative humidity, as well as of processing area and methods used during processing to pre and post-harvest practices, are also the main cause and sources for fungal association [27].

Although it is difficult to remove mycotoxins from maize grain or crops, it is possible to reduce the risk of exposure through a rigorous program of monitoring these toxins in crops and feeds through appropriate drying and storing method.

Monitoring of toxigenic fungi and mycotoxins in crops is especially important because it not only provides a healthier diet for man, but it also may indirectly prevent any mycotoxin residue carryover in animals for human consumption. Poor post-harvest management can lead to rapid deterioration in the nutritional quality of seeds e.g maize. Microbial activity can cause undesirable effects in grains including discoloration, contribute to heating and losses in dry matter content through the utilization of carbohydrates as energy sources, degrade lipids and proteins or alter their digestibility, produce volatile metabolites giving off-odours, cause loss of germination and baking and malting quality; Filamentous fungal spoilage organisms may also produce mycotoxins that can be carcinogenic or cause food/feed refusal.

Therefore, the aim of the research is to;

- Study the spore level of toxigenic Aspergillus flavus inoculated on a maize grain during drying using different methods.
- Determine the best and most efficient method of drying maize grains using locally fabricated solar dryer lined with aluminium foil, solar dryer lined with black polythene, tray lined with plantain (Musa paradisiaca) leaf, tray lined with Thaumatococcus danielli leaf and calabash that will reduce the sporulation level of the toxigenic mold Aspergillus flavus.
2. MATERIALS AND METHODS

2.1 Collection of Samples

Yellow maize grains were obtained from a maize farm at Ilaara-mokin, Ondo state, Nigeria. Culture of toxigenic Aspergillus flavus was isolated from poultry feed sample and maintained on Czapek Dox Agar. Wooden solar dryers were locally fabricated and the surfaces were lined with different materials like foil paper, polythene nylon to compare the best heat reflecting or absorbing surfaces, different trays were also lined with various leaves; Thaumatococcus daniellii and plantain (Musa paradisiaca) leave to determine the best traditional method of drying using leaves.

2.2 Isolation of Toxigenic Aspergillus flavus

Colonies of Aspergillus species were transferred to 5–2 agar slant and incubated for three days after which they were purified by single spore isolation on 5–2 agar plate. The emerging colonies were sub-cultured by a three-point inoculation on Czapek’s–Dox (CZ) agar and incubated at 25°C for identification [28,29].

2.3 Screening of Toxigenic Aspergillus flavus isolates

Aspergillus flavus strains that do produce sclerotia are significantly likely to produce aflatoxins [30] therefore, only single spore isolates of A. flavus that produced sclerotia were assessed for the ability to produce a beige ring in culture using the method described by Ordaz et al. [31]. The isolates were grown in duplicate on Yeast Extract Sucrose (YES) constituted by 20 g yeast extract (Merk, Darmstadt, Germany), 150 g Sucrose (Merk, Darmstadt, Germany), 20 g agar (Oxoid, Bakingstoke, UK) supplemented with 0.3% wt/v methyl β-cyclodextrin (Sigma-Aldrich, Steinheim, Germany) and 0.6% wt/v Sodium deoxycholate (Sigma-Aldrich, Steinheim, Germany), and incubated at 28°C for 7 days in the dark.

2.4 Inoculation of Toxigenic Aspergillus flavus in the Maize Grains

Maize grains were weighed into sterile resealable bags. About 305 g of maize was weighed into each resealable bag respectively and soaked with water for 6 hours to soften the cotyledons. After 6 hours, the water was decanted from the maize and autoclaved at 121°C for 15 minutes. Spore suspension from the sub-cultured isolate was prepared under inoculating chamber by adding 20 mL of sterile water containing two drops of tween 20 to culture media used for the isolation of A. flavus [32]. Spores suspension was harvested and counted with hemocytometer counting chamber by counting 10⁶ spu/ml respectively from 20 mL spore suspension and thereafter dispensed into the maize grains in each resealable bag, the mixture were mixed thoroughly and incubated at room temperature for 18 hours for mycelium growth [32]. The interval used for counting the spore in the inoculating chamber was 7 days to allow the mould to sporulate. After incubation for 18 hours, the inoculated maize grain were dried using different drying methods (Table 1).

Table 1. Various drying method used

<table>
<thead>
<tr>
<th>SN</th>
<th>Drying method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Solar dryer lined with aluminium foil</td>
</tr>
<tr>
<td>2</td>
<td>Solar dryer lined with black polythene</td>
</tr>
<tr>
<td>3</td>
<td>Tray lined with Thaumatococcus daniellii</td>
</tr>
<tr>
<td>4</td>
<td>Tray lined with plaintain (Musa paradisiaca) leave</td>
</tr>
<tr>
<td>5</td>
<td>Calabash (Crescentia cujete)</td>
</tr>
</tbody>
</table>

2.5 Incubation and Estimation of Sclerotia Spores Produced by Aspergillus flavus

Humidity chamber was used for isolation and production of sclerotia by using a sterile petri dish lined with sterile absorbent cotton wool soaked with about 4 mL of sterile water, covered with a single layer of filter paper and sterilized in the autoclave. Thereafter, nine maize grains samples from the various drying methods were arranged on the moist filter paper 3 by 3 with the aid of a sterile forceps (Plate 1). The plate were covered and incubated at 28°C and the spores colonies on the maize were counted using haemocytometer counting chamber with the aid of microscope after 7 days of incubation by picking five incubated maize grain diluted in 50mLs of water containing a drop of tween 20 [33].
2.6 Determination of Moisture Content

The samples moisture content was expressed as a percentage. The percentage of moisture content determined by conventional oven method was calculated using the following equation [34].

\[ \%W = \frac{(A - B)}{B} \times 100 \]

Where:

\%W = Percentage of moisture in the sample,  
A = Weight of wet sample (grams), and  
B = Weight of dry sample (grams)

2.7 Data Analysis

The experiment was conducted using a completely randomized design. Means of three replicates were computed using computer software Microsoft Excel.

3. RESULTS

3.1 Evaluation of the Growth of the Isolate Subcultured

After 48 hours of incubation, visible small whitish cotton like mycelia was observed around the point of inoculation. After a week the organism has grown into a brownish numerous spores, with production of brownish liquid/ spores called sclerotial. As the day and hour of incubation increases more growth occurred with more obvious brown spore growing into a star like shape colony and production of sclerotial, as seen in Plate 2.

3.2 Estimation of the Number of Spores Counted at day 1-3 During Drying with Different Drying Technique

The mean value was recorded for total number of spores counted from the maize incubated in inoculating chamber with the initial spore suspension of $10^6$ sfu/g and stored for counting interval of seven days respectively (Table 2 and Figs. 1-3). There was increase in the spores level...
of maize dried in calabash to $9.85 \times 10^6$ sfu/g on day 1 of drying at the moisture content of 84.35% but reduction was observed on day 2 ($6.3 \times 10^6$ sfu/g) at a reduced moisture content of (21%) and finally reduced to $9.50 \times 10^5$ sfu/g at 14% moisture content.

There was reduction in the spores level of maize dried in tray lined with *Thaumatococcus daniellii* leaf to $4.2 \times 10^6$ sfu/g on day 1 of drying at the moisture content of (88.35)% and further reduction was observed on day 2 ($1.95 \times 10^6$ sfu/g) at moisture content of (20%) and later increased to $2.35 \times 10^6$ sfu/g at moisture of 15%.

Reduction was observed in the spores level of maize dried in tray lined with plantain leaf to $5.90 \times 10^6$ sfu/g on day 1 of drying at the moisture content of (85, 34) and further reduction was observed on day 2 ($2.05 \times 10^6$ sfu/g) and day 3 ($1.15 \times 10^6$ sfu/g) respectively at moisture content of (20, 13)%

Reduction was also observed in the spores level of maize dried on solar dryer lined with aluminum foil to $3.6 \times 10^6$ sfu/g on day 1 of drying at the moisture content of (80, 32) and further reduction was observed on day 2 ($9.20 \times 10^5$ sfu/g) and day 3 ($5.50 \times 10^5$ sfu/g) respectively at moisture content of (15, 13)%.
There was increase in the spores level of maize dried on dryer lined with black polythene to $7.00 \times 10^6$ sfu/g on day 1 and day 2 ($8.10 \times 10^6$ sfu/g) of drying at the moisture content of (80,34) and later reduction was observed on day 3 ($2.00 \times 10^6$ sfu/g) at moisture content of (14%). Fluctuation occur in the sporulation level of A. flavus from maize grain dried with Thaumatococcus daniellii leaf, solar dryer lined with aluminum foil and solar dryer lined with black polythene method. The value of counted spores of all weekly intervals from maize grain dried with solar dryer lined with black polythene method is lower than that of solar dryer lined with aluminum foil, this observation shows that solar dryer lined with aluminum foil is more efficient in reducing the toxigenic spores of A. flavus than solar dryer lined with black polythene.

3.3 Moisture Content of Maize Seeds

The moisture content of the maize samples ranges from 80%-13%) (Fig. 4). From the result obtained it was observed that reduction was observed in the percentage moisture contents of all the samples. The highest reduction was observed in maize sample dried on Solar dryer lined with aluminum foil and Tray lined with plantain leaf. Also comparing the effect of moisture on the growth performance of the organism in the maize during drying, it was observed that highest reduction occurred in sample dried with Solar dryer lined with aluminum foil which also had the least moisture content.

4. DISCUSSION

This was a case study carried out in order to determine the influence of different drying methods on the rate of sporulation of toxigenic Aspergillus flavus on a maize grains. When cultured on the media, the first visible growth of the Aspergillus flavus occurred after 48 hours of incubation at room temperature (25°C). The growth seen after 48 hours was a visible whitish mycelia growing at the point of inoculation, after the mycelia growth, spores were produced alongside the sclerotia which is linked with the observation of [35].

The maize (Zea mays) grain used in this study was a yellow dent maize species. The main features that distinguish this from other types of corn is that during drying, the soft endosperm collapses to form an indentation or dimple and this observation was seen when the maize grain subjected to drying. Due to the soft endosperm dents, this type of corn is more susceptible to grain insects and molds, both in the field and in storage [36].

In this study, a relatively high level of spores (sfu) of A. flavus that was observed in the maize dried with calabash compared with the result of spores level of other materials used. This can be compared with the report of Cardwell & Cotty [37] when he conducted a research on inoculated A. flavus propagules in an area, recording some samples exceeding 5000 cfu/g, while the average inoculation was around 486 cfu/g. The
Table 2. Showing the mean value of spores counted at weekly interval using haemocytometer counting chamber

<table>
<thead>
<tr>
<th>SN</th>
<th>Drying method</th>
<th>Initial inoculated spore number</th>
<th>Total no of spores (sfu/ml) counted at day 1 of drying</th>
<th>Total no of spores (sfu/ml) counted at day 2 of drying</th>
<th>Total no of spores (sfu/ml) counted at day 3 of drying</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calabash</td>
<td>6,000,000</td>
<td>9,850,000</td>
<td>6,300,000</td>
<td>950,000</td>
</tr>
<tr>
<td>2</td>
<td>Tray lined with <em>Thaumatococcus daniellii</em> leaf</td>
<td>6,000,000</td>
<td>4,250,000</td>
<td>1,950,000</td>
<td>2,350,000</td>
</tr>
<tr>
<td>3</td>
<td>Tray lined with plantain leaf</td>
<td>6,000,000</td>
<td>5,900,000</td>
<td>2,050,000</td>
<td>1,150,000</td>
</tr>
<tr>
<td>4</td>
<td>Solar dryer lined with aluminium foil</td>
<td>6,000,000</td>
<td>3,600,000</td>
<td>5,300,000</td>
<td>550,000</td>
</tr>
<tr>
<td>5</td>
<td>Solar dryer lined with black polythene</td>
<td>6,000,000</td>
<td>7,000,000</td>
<td>8,100,000</td>
<td>2,000,000</td>
</tr>
</tbody>
</table>

Fig. 4. Moisture content of the samples

The growth level of *A. flavus* sfu of the dried maize seeds indicated that infection took place at two major stages. There was an initial decrease in sfu level from the first to the third day of drying. The high levels of spore increase that developed during the day 1 in the calabash indicated that *A. flavus* contamination already occurred in the drying container prior to drying, which is in agreement with previous reports.
5. CONCLUSION

In conclusion, this study demonstrates the role of primary inoculum on A. flavus infection and the rate of colonization of grains, and how they affect production of toxigenic A. flavus in maize during drying using different method of drying and also documents the effect of percentage moisture on the growth of toxigenic A. flavus in maize. For the proper drying of maize grain, environmental factors such as temperature and moisture content must be controlled. Such factors are the major influences of maize deterioration, because they affect molds, insects, and other pest, which can result in huge losses of maize grain in a very short time. To avoid mycotoxin contamination, maize should be monitored regularly to assure safe storage conditions, hence, maize contaminated by fungi and molds not only render grains unfit for human consumption by discoloration, but can also lead to toxin production such as aflatoxins and fumonisins which are harmful to human and animal health.

Considering the different materials used for drying maize in this study, Thaumatococcus danielli leaf, Musa paradisiaca and Calabash drying, Calabash and solar dryer lined with aluminum foil proves to be more reliable and efficient in reducing the sporulation level of A. flavus than other material used.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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