Detection of fungi in stored maize grain:
Isolation, identification and characterisation in Birnin Kebbi, Nigeria

BJ Danjumma, DN Peni, M Yusuf and AG Benedict

DOI: https://doi.org/10.22271/j.en.to.2022.v10.i5a.9049

Abstract
The study isolate, identified and characterized fungi associated with spoilage of store maize grains in Birnin Kebbi. Two maize varieties were used samar 37VA and samar 37B. The two samples were collected from the store for isolation and identification of fungi associated with maize grain spoilage. Pour plate method were used in the enumeration of fungi which showed that fungi count was found on samar 37 VA washed maize had $4\times 0.00$ to $8\times 1.11$ and unwashed samar 37 VA had $7.00\pm 1.00$ to $10\times 3.00$. Pure colonies on PDA agar plate were identified base on the morphological characteristics. Twenty nine (29) fungi isolates were identified and belong to three (3) genera i.e Aspergillus species, Rhizopus species and Penicillium species. Inferential statistics were used (Frequency and percentage). Aspergillus species had 13(44.83), Rhizopus species 11(37.93), Penicillium species 5 (17.24). Aspergillus sp. Were the most frequent fungi isolated. From the study it showed that maize grain are affected by fungi under various treatment factors. Therefore there is the need to further study the biochemical and molecular properties of the fungi isolated and to screen the fungi isolated for cellulytic properties.

Keywords: Maize, fungi, isolation, characterization and spoilage

Introduction
In Africa Maize (Zea mays L.) are most grown cereal and consumed cereal crops followed by wheat, sorghum and rice (Danho et al., 2002) [4]. It covered small land area than other cereal crops produced with high yield per unit area of about 5.5 tonnes per hectare (Ofori et al., 2004) [10]. The maize grain had 70-72% digestible carbohydrate, 4 - 4.5% fats and 9.5-11% proteins (Ofori et al., 2004) [15].

In many countries about 66% of maize is used for formation of livestock feed, human consumption used 25% and 9% for other purposes (Danho et al., 2002) [4]. Maize (Zea mays) is predominantly grown in Kaduna, Niger, Jos, Benue, Nasarawa, Kebbi and other part of Nigeria (Danjumma et al., 2018) [8]. The fungi spoilage reduces the availability of maize to consumers thereby causing economic loss to the farmers. Spoilage of maize reduces the nutritional, minerals and vitamins contents as well, increasing its allergic potential value and palatability of the feed (Danjumma et al., 2018) [8].

Postharvest losses commonly take place during storage of maize grain; the maize grain is infected by entomo parasites, predators and microorganisms (Neethirajan et al., 2007) [13]. Such infestations cause a reduction in product quality and economic loss (Birck et al., 2003; 2006) [1, 2]. fungi and their associated secondary metabolites known as mycotoxins are of high concern in grain shipments or storage facilities due to the production of mould, odours, the presence of microbial ‘hot-spots’, and the production of secondary metabolites which can lead to subsequent poisoning of food and animal feed, thus negatively impacting food safety (Tefera et al., 2011) [14].

There are a number of postharvest fungi that can attack and cause damage to maize, and they can be classified as: field fungi and storage fungi (Miller, 1995). Field fungi may modify the structure and quality of seeds or grains (Chelladurai et al., 2010). These cause damage to the grain before harvest and can generally be detected by routine assessment. In general, field fungi do not occur in storage if the grain is stored at appropriate moisture contents and temperatures (Miller, 1995) [10]. Storage fungi are those that cause damage to grain during storage and usually do not occur at a serious level prior to harvest (Muir and White 2000) [12].
Mycotoxins produced by some fungi cause a large number of diseases annually and it affected some vital organs and system of human body (liver and Respiratory and gastro intestinal tract), cause acute toxicosis, immune disorder and growth dysfunction in children. The majority of infections of animals (e.g chronic aflatoxicoses) on farms are caused by mycotoxins being present in poor quality feed (Zain, 2011) [8]. For example, Aflatoxin B1 is highly toxic and is a potent carcinogen to both humans and animals. *Fusarium moniliforme* produced Fumonisins B1 (FB1) associated with equine leukoencepha-lomalacia and porcine pulmonary edema (Kellerman et al., 1990, Harrison et al., 1990) [8, 9]; these infections were observed in livestock after they had consumed spoiled maize grain. Durin storage period maize grains are associated with fungi spoilage in Birnin Kebbi causing shortage and economic loose. Lack of information on fungi associated with storage maize grain. Hence this prone the research work to isolate and identify fungi associated with storage maize in Birnin Kebbi North western part of Nigeria.

**Methodology**

**Description of the Study Area**
The study was carried out in Birnin Kebbi North western Nigeria located between latitude 10°N and 15.5°N and longitude 3°W and 6°W. The climate of the area is generally characterized by high temperature ranging between March and May with mean annual temperature 38 °C and 41 °C and the area experiencing harmattan wind between late November to early February, with temperature as low as 23 °C.

**Sample Collection, Media preparation, enumeration, isolation and identification of fungi colonies**
The two varieties of Maize grain were collected from ministry of Agriculture incubation center stores in Birnin Kebbi North western Nigeria. The media (PDA) for culturing were aseptically prepared when needed according to the manufacturer’s instruction and autoclaved at 121 °C for 15 min as described by. Six (6) fold serial dilutions of the sample were prepared. The diluted samples were used to inoculate the prepared media using pour plate method. The agar plates were allowed to solidify and placed in an inverted position for 5-7 days at 37 °C, thereafter, their colonies were observed and counter in triplicate as described by Yusuf et al. (2018) [17]. The growth pattern, pigmentation and size of colonies were recorded at the incubation period to aid identification of the fungi Danjumma et al. (2019) [9]. A drop of lactophenol (LP) was placed on a clean microscopic slide. A small portion of the isolate was placed in the drop of lactophenol (LP) and suspended. A clean cover glass was placed over the suspension and observed microscopically Danjumma et al. (2019) [9].

**Results and Discussion**
The fungi isolated from two (2) maize varieties samples together with their enumeration shown in Tables 1. Higher fungi count was found on samar 37 VA unwashed maize had 7±1.00 to 10± 3.00 with less fungi count on washed samar 37 VA. With 4.00±0.00 to 8±1.11. Microscopically and morphological features (cell size, shape, pigmentation and arrangements) of colonies were used for the isolation and identification. The results were presented in Table 1. The cultural morphology revealed that many of the fungi appeared to be black, white, dark green, yellow and white cottony on PDA agar plate incubated for 5 to7 days at 37°C. The isolated fungi belong to three (3) genera. The fungi isolated were *Aspergillus sp*, *Rhizopus sp* and *Penicillium sp*. This work is similar with the work of Onyeeze et al. (2013) [16].

Table 1: Showed the enumeration, cultural, microscope and suspected fungi from various treatment

| Sample maize | Treatment | T0 cfu/ml | Cultural morphology | Microscope features | Suspected fungi |
|--------------|-----------|-----------|---------------------|---------------------|----------------|
| Samar 37 VA  | Washed    | 4+000     | Black               | Conidial heads were large globose, dark brown which become radiate | Aspergillus specie |
| Samar 37 VA  | Washed    | 8+1.11    | Black               | Conidial heads were large globose, dark brown which become radiate | Aspergillus sp |
| Samar 37 VA  | Unwashed  | 10+3.00   | White cotton        | Sporangiospores were small. Round and oval                  | Rhizopus specie |
| Samar 37 VA  | Unwashed  | 7+1.00    | Grey                | Flask shaped                                               | Penicillium sp |
|              |           |           | Yellow              | Conidial head were observed                               | Aspergillus specie |
|              |           |           | Yellow              | Conidial head were observed                               | Aspergillus specie |
|              |           |           | White cotton        | Sporangiospores were small. Round and oval                  | Rhizopus sp |
|              |           |           | Black               | Conidial heads were large globose, dark brown which become radiate | Aspergillus specie |
|              |           |           | Yellow              | Conidial head were observed                               | Aspergillus specie |
|              |           |           | White cotton        | Conidial heads were observed                               | Aspergillus specie |
|              |           |           | White cotton        | Sporangiospores were small. Round and oval                  | Rhizopus sp |
|              |           |           | Yellow              | Conidial head were observed                               | Aspergillus specie |
|              |           |           | White cotton        | Conidial heads were observed                               | Aspergillus sp |
|              |           |           | Yellow              | Conidial head were observed                               | Aspergillus specie |
|              |           |           | Yellow              | Conidial head were observed                               | Aspergillus specie |
|              |           |           | White cotton        | Conidial heads were observed                               | Aspergillus specie |

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The results showed that Aspergillus species are the most frequent fungi isolated in the samples analyzed. The processing method are the major factors associated with fungi occurrence in stored maize. In adequate processing methods (drying method) are responsible for fungi growth. Aspergillus genera need moisture for their growth (Harrigan, 1988). Aspergillus species produces diverse toxigenic lineage in maize and it derived products (Larone, 1998). The results shows that Aspergillus species had 13(44.83%), Rhizopus species had 11(37.93) and Penicillium species had 5(17.24%) (Table 2, 3 and figure1). The results show that Aspergillus species, Rhizopus species and Penicillium species are fungi that associated with the spoilage of stored maize grain in the study area. The toxin produced by these genera of fungi are serious threats in human health and causing a public health concern. The results conclude that maize grains are associated with spoilage by fungi following recommendation were drawn from the results obtained.

1. Further biochemical and molecular study should be done on the fungi isolated
2. The isolated fungi should be screen for cellulolytic properties.

| Table 2: Frequency and Percentage Occurrences of fungi isolated from various maize varieties and treatment |
| Sample maize | Treatment | Suspected fungi | Frequency | Percentage |
| Samar 37 V A | washed | Aspergillus species | 1 | 25 |
| Samar 37 V B | washed | Rhizopus species | 3 | 75 |
| Samar 37 V A | unwashed | Penicillium species | 4 | 50 |
| Samar 37 V A | unwashed | Rhizopus species | 4 | 50 |
| Samar 37 V A | unwashed | Aspergillus species | 6 | 60 |
| Samar 37 V B | washed | Rhizopus species | 3 | 30 |
| Samar 37 V B | washed | Penicillium species | 1 | 10 |

| Fungi isolated | Frequency | Percentage (%) |
|----------------|-----------|----------------|
| Aspergillus sp | 13 | 44.83 |
| Penicillium sp | 5 | 17.24 |
| Rhizopus sp | 11 | 37.93 |

Fig 1: Pie chart showed the percentage of fungi isolated

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