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

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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± 0.00 to 8± 1.11 and unwashed samar 37 VA had 7.00±1.00 to 10± 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 cellulolytic 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) [15]. 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) [5]. 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) [5].

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 grain, 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) [11]. 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 Respitatory 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) [18]. For example, Aflatoxin B1 is highly toxic and is a potent carcinogen to both humans and animals. *Fusarium moniliforme produced* Fumonisin B1 (FB1) associated with equine leucoencepha-lomalacia and porcine pulmonary edema (Kellerman *et al*., 1990, Harrison *et al*., 1990) [9, 8]; these infections were observed in livestock after they had consumed spoilt 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 10º and 13.5º N and longitude 3º and 6º W. The climate of the area is generally characterized by high temperature ranging between March and May with mean annual temperature 38 ºC and 41 ºC and the area experiencing harmattan wind between late November to early February, with temperature as low as 23 ºC.

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 ºC 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) [6]. 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) [6].

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 37ºc. The isolated fungi belong to three (3) genera. The fungi isolat ed were *Aspergillus sp, Rhizopus sp* and *penicillium sp*. This work is similar with the work of Onyeze *et al*. (2013) [16].

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

Sample maize	Treatment	10^4cfu/ml	Cultural morphology	Microscope features	Suspected fungi
Samar 37 V A	Washed	4+00	Black	Conidial heads were large globose, dark brown which become radiate	*Aspergillus specie*
			White cottony	Sporangiospores were small. Round and oval	*Rhizopus specie*
			Dark green	Radiate conidial head were observed	*Aspergillus specie*
			Black	Conidial heads were large globose, dark brown which become radiate	*Aspergillus specie*
			Grey	Flask shaped	*Penicillium specie*
			Dark green	Radiate conidial head were observed	*Aspergillus specie*
			White cottony	Sporangiospores were small. Round and oval	*Rhizopus specie*
			Yellow	Conidial head were observed	*Aspergillus specie*
			White cottony	Sporangiospores were small. Round and oval	*Rhizopus sp*
			Black	Conidial heads were large globose, dark brown which become radiate	*Aspergillus sp*
Samar 37 V B	Washed	8+1.11	Black	Conidial heads were large globose, dark brown which become radiate	*Aspergillus sp*
			Black	Conidial heads were large globose, dark brown which become radiate	*Aspergillus sp*
			Yellow	Conidial head were observed	*Aspergillus sp*
			Dark green	Radiate conidial head were observed	*Aspergillus sp*
			Dark green	Radiate conidial head were observed	*Aspergillus sp*
			White cottony	Sporangiospores were small. Round and oval	*Rhizopus sp*
			Yellow	Conidial head were observed	*Aspergillus sp*
Samar 37 V A	Unwashed	10+3.00	White cottony	Sporangiospores were small. Round and oval	*Rhizopus sp*
			Yellow	Conidial head were observed	*Aspergillus sp*
			Yellow	Conidial head were observed	*Aspergillus sp*
			Yellow	Conidial head were observed	*Aspergillus sp*
Samar 37 V B	Unwashed	7+1.00	Grey	Flask shaped	*Penicillium sp*
			White cottony	Sporangiospores were small. Round and oval	*Rhizopus sp*
			White cottony	Sporangiospores were small. Round and oval	*Rhizopus sp*

			White cottony	Sporangiospores were small. Round and oval	*Rhizopus sp*
			Grey	Flask shaped	*Penicillium sp*
			Grey	Flask shaped	*Penicillium specie*
			Grey	Flask shaped	*Penicillium specie*
			White cottony	Sporangiospores were small. Round and oval	*Rhizopus specie*

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 occurance in stored maize. In adequate processing methods (drying method) are responsible for fungi growth. *Aspergillus* genera need moisture for their growth (Harrigan *et al*., 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 produces 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 prope rties.

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		*Rhizopus species*	3	75
		Penicillium species	4	50
Samar 37 V A	unwashed	*Rhizopus species*	4	50
		Aspergillus species	6	60
		Rhizopus species	3	30
		Penicillium species	1	10
Samar 37 V B		*Aspergillus species*	6	85.71
		Rhizopus species	1	14.29

Table 3: Percentage of the three (3) species of fungi isolated

Fungi isolated	Frequency	Percentage (%)
Aspergillus sp	13	44.83
Penicillium sp	5	17.24
Rhizopus sp	11	37.93
	29	100

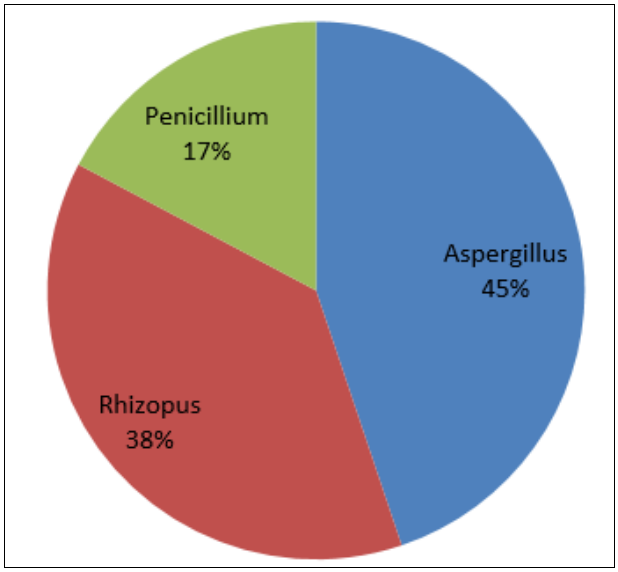

Fig 1: Pie chart showed the percentage of fungi isolated

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