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Research Article

Determination of Fungal Population in Maize Grains from Benue State Nigeria

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ABSTRACT

Determination of fungal population in maize grains collected from Benue State was carried out in this study. One hundred and seventeen maize grain samples purchased from markets in Benue State were screened for toxigenic and non-toxigenic fungal contaminants. Maize samples (500 g each) were collected in sterile polythene bags, labelled and taken to the laboratory for analysis. Isolation and identification of the fungi was carried out using dilution method and standard mycological procedures. Results showed that almost all the seed samples (99.1%) were contaminated. Many (55.6%; 65/117) of the maize grains were moderately contaminated with 1.0 × 06 to 2.7 × 107 colony forming units per gram (cfu/g) of sample, while few (7.1%; 9/117) showed high level of fungal contamination (>8.2 × 107 cfu/g). Many samples 42.7% (n=50/117) were free of contamination with *Rhizopus stolonifer*. However, 41.9% (n=49/117) showed some *Rhizopus stolonifer* contamination (1.0 × 10^6 to 2.5 × 107 cfu/g). Exactly 40.2% (n=47/117) of the seed samples were free of *Aspergillus* contamination, while 34.2% (n=40/117) showed some level of contamination (1.0 × 10^6–2.0 × 107 cfu/g). Only few (2.6%; n=3/117) were highly contaminated (>6.1 × 107 cfu/g) with *Aspergillus*. A high number (91.5%; n=107/117) of the maize samples were free of contamination with *Penicillium notatum*, very few 2.6% (n=3/117) of the samples were lightly contaminated (1.0 × 10^6–1.0 × 107 cfu/g). Moderate contamination (1.1 × 107 – 2.0 × 107 cfu/g) was observed for 4.3% (n=5/117) of the maize samples. Majority of the seeds (73.5%, n=86/117) were free of fungal *Fusarium* contamination, while only a few (4.5%; n=5/117) were heavily contaminated (≥ 4.8 × 107). Due to the high level of fungal contamination found in this study, there may be need for emphasis on education of the populace on the proper method of harvesting, drying, handling, transportation and storage of maize grains by relevant authorities.

Keywords: Fungi; Maize grain; Contamination; Aflatoxin; Benue state

INTRODUCTION

Fungi are responsible for the production of secondary metabolites called mycotoxin [1]. Mycotoxins are mainly produced by fungal species belonging to the genera *Aspergillus*, Penicillium and *Fusarium* which are ubiquitous in the environment [2,3]. Infection of plants by fungi causes low crop yield and quality resulting in catastrophic economic losses [4]. However, ingestion of contaminated grains with aflatoxin has intense public health significance because these toxins have nephrotoxic, immunotoxic, teratogenic, carcinogenic and mutagenic consequences [4-6].

Maize is a crop grown in Nigeria for both human food consumption and livestock feeding [7]. It is significantly implicated in severe mycotoxin problems because of its susceptibility to colonization by aflatoxin producing *Aspergillus* species [8]. Kaaya and Kyamuhangire [9] reported the presence of *Aspergillus, Fusarium* Penicillium and *Rhizopus* species and high levels of AFB1 in maize. Aflatoxins are produced by fungal action during production, harvest, transportation, storage and food processing [10,11]. Aflatoxin contaminated food on the field and during storage has economic significant [12]. Previous studies have proposed that the occurrence of fungi and aflatoxins in food products is mainly influenced by favorable conditions such as high moisture content and temperature [13]. In countries with food legislation, fungal contaminated foods are considered illegal and not acceptable in trade therefore constituting huge losses and health hazard [14]. Therefore, the detection of aflatoxin producing fungi is very important in Nigeria. This study investigates the incidence of fungal food borne pathogens in Benue State.

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MATERIALS AND METHODS

Sample collection

Maize grains were purchased from local markets in sterile polythene bags, labelled and tied separately according to the sample locations. They were immediately transported to Microbiology Laboratory University of Agriculture Makurdi for mycological identification and biomass. Multi-stage sampling was used. The choice of market from which samples were taken was based on the location of the market, the number of maize sellers in the area and popularity of the maize grain market within the study area. Maize grain samples were collected randomly from markets in Benue State and a total of 117 maize samples (500 g each) were collected.

Mycological identification

Potato Dextrose Agar (PDA) medium was prepared according to manufactures method. Samples collected from each Local Government Area were analyzed for mycological contamination. Briefly, under aseptic conditions, samples (500 g) were finely ground to obtain a homogeneous mixture using the commercial blender and 1 g from each sample was mixed with sterile distilled water (9 mL) followed by ten folds serial dilution. One millilitre from each dilution was then transferred to the Petri-dishes and a cooled medium spread on top and mixed gently for 2 min. Isolation of mycoflora was done by direct plating of ground samples onto plates of Potato Dextrose Agar (PDA) supplemented with 60 ug ml-1 chloramphenicol as a bacteriostat [15-17]. The mixture was allowed to solidify and then incubated at 30° C for 5-7 days. The suspected mycotoxigenic fungi colonies were further purified individually by sub culturing on PDA plate and then on PDA slant (15 ml slant bottle). Isolated fungi were then identified according to Raper, Fennel, Nelson, Rechard and Klich, based on colony characteristics and morphology under light microscope [18-21].

Statistical analysis

Data was subjected to Analysis of Variance (ANOVA) and Chi square test using GENSTAT statistical software (17th edition). Descriptive statistics was also used. Statistically significant means was separated using F-LSD at 5% level of probability.

RESULTS AND DISCUSSION

One hundred and seventeen maize grain samples purchased from thirteen Local Government Areas of Benue State were screened for toxigenic and non-toxigenic fungal contaminants. Results showed tested maize grains samples and got total fungal count of 1×10^1 to that almost all the seed samples (99.1%) were contaminated. Many (n=65; 55.6%) were moderately contaminated with 1.0×10^6 to 2.7×10^7 colony forming units per gram (cfu/g) of sample and few (n=9, 7.1%) showed high level of fungal contamination ($\geq 8.2 \times 10^7$ cfu/g) (Table 1). The rest (19.7% and 16.2%) had fungal counts ranging from 2.8×10^7 to $5.4\ 10^7$ cfu/g of sample. Similarly, Krnjaja, 3×10^6 cfu/g [22]. In another study, [23] analyzed 30 samples of maize grains collected from different locations and got total fungal count of 5×10^5 to 5.2×10^7 cfu/g. Maize is considered as one of the best substrate for fungi to grow and produce toxicogenesis [24].

Table 1: Frequency of seeds contaminated by toxic and non-toxic fungal contaminants.

No. of fungal colony forming units (cfu/g of seed)	Frequency	Percent (%)
≤ 1.00	1	0.9
1.0×10^6 - 2.7×10^7	65	55.6
2.8×10^7 - 5.4×10^7	23	19.7
5.5×10^7 - 8.1×10^7	19	16.7
8.2×10^7 - 1.00×10^8	9	7.1
Total	117	100

According to reports of FAO (1983) maize grain is a better substrate for fungal growth than most crops. Bankole and Mabekoje reported that maize is likely to have higher moisture content than other crops [25]. Although moisture content was not determined in this work, it may be responsible for the high rate of fungal contamination observed in this study. In the work done by Krnjaja [22], there was a positive correlation between the moisture contents of maize samples and the total fungal count. Previous studies have proposed that the occurrence of fungi and aflatoxins in food products is mainly influenced by favorable conditions such as high moisture content and temperature [13].

Extent of maize grain contamination with *Rhizopus stolonifer* observed in this study showed that, many samples (42.7%; n=50) were free of contamination with *Rhizopus stolonifer* (Table 2). However, 41.9% (n=49) showed moderate Rhizopus contamination (1.0×10^6 to 2.5×10^7 cfu/g). This is contrary to reports of Alptekin, Krnjaja [22, 23], Rhizopus contamination was not found in their study. It was also observed in this study that many (91.5%; n=107) of the maize samples were free of contamination with *Penicillium notatum* and *Fusarium* vulani (73.5%, n=86) (Tables 3-6). On the contrary, Covarelli [26] analyzed maize grain samples from different locations and isolated *Fusarium* species with the highest occurrence (76.8%), followed by *Aspergillus* with 14.5% and Penicillium species with 9.2%. Likewise isolated most common fungi to be *Fusarium* (31.74%), then *Aspergillus* genera (30.83%), followed by Penicillium (13.75%) and Alternaria species (1.66%) [27]. Environmental factors such as temperature and water activity contributes significantly to the type of fungal species prevalent in a given location [28]. This may be responsible for variation in contamination levels of the maize grains observed in this study.

Table 2: Rate of Maize Grain contamination with *Rhizopus stolonifer* in Benue State.

No. of colony forming units/gram of seed of *Rhizopus* species	Frequency	Percent (%)
≤ 0.00	50	42.7
1.0×10^6 - 2.5×10^7	49	41.9
2.6×10^7 - 5.0×10^7	13	11.1
5.1×10^7 - 7.5×10^7	3	2.6
≥ 7.6×10^7	2	1.7
Total	117	100.0

Table 3: Rate of Maize Grain contamination by *Aspergillus* species in Benue State.

No of colonies (cfu/g)	Frequency	Percent (%)
≤ 0.00	47	40.2
1.0×10^6 –2.0×10^7	40	34.2
2.1×10^7–4.0×10^7	19	16.2
4.1×10^7– 6.0×10^7	8	6.8
≥ 6.1×10^7	3	2.6
Total	117	100.0

Table 4: Rate of Maize Grain contamination with *Penicillium notatum* in Benue State.

No of colonies (cfu/g)	Frequency	Percent (%)
≤ .00	86	73.5
1.0×10^6 – 2.3×10^7	21	17.9
2.3×10^7 – 4.7×10^7	5	4.3
≥ 4.8×10^7	5	4.3
Total	117	100.0

Table 5: Rate of Maize Grain contamination by *Fusarium* vulani in Benue State.

No of colonies (cfu/g)	Frequency	Percent (%)
≤ .00	107	91.5
1.0×10^6 – 1.0×10^7	3	2.6
1.1×10^7 – 2.0×10^7	5	4.3
≥ 2.1×10^7	2	1.7
Total	117	100.0

Table 6: Percentage occurrence of fungal colonies found in maize grains in Benue State.

Fungi species	Frequency	Percentage (%)
Aspergillus present	70	59.8
Aspergillus absent	47	40.2
Total	117	100
Rhizopus stolonifer present	67	57.3
Rhizopus stolonifer absent	50	42.7
Total	117	100
Penicillium notatum present	10	8.5
Penicillium notatum absent	107	91.5
Total	117	100
Fusarium vulani present	31	26.5
Fusarium vulani absent	86	73.5
Total	117	100

The common fungi isolated in this study were; *Aspergillus* species (59.8%), *Rhizopus stolonifer* (57.3%), *Penicillium notatum* (8.5%) and *Fusarium* vulani (26.5%). Many of these fungi have also been found to cause spoilage in maize in Nigeria and other parts of the world. Similarly, reports from other studies had identified *Aspergillus* species as the most predominant mycotoxigenic fungi. Kamika and Takoy also stressed that many food crops like maize and groundnut are susceptible to contamination by aflatoxigenic fungi. Similarly, Yilma [29] reported 50.7% incidence rate of *Aspergillus* species in maize. Chauhan and Tassew [30] also reported high frequency of occurrence of *Aspergillus* species. The high frequency of occurrence of *Aspergillus* species observed in these locations could be due to poor storage and longer storage time [30-33] Gbodi identified the following fungi in maize; *Fusarium* species, *Fusarium* moniliforme, Helminthosporium species, Penicillium species, Phoma sorghina species and Rhizopus species among others [34]. In a similar survey, Kaaya and Kyamuhangire [9] also reported the presence of *Aspergillus*, *Fusarium*, Penicillium and Rhizopus and high levels of AFB1 in maize. In another survey, Krnjaja [22] revealed potentially toxigenic fungi (*Aspergillus, Fusarium* and Penicillim genera) as the most common in all the samples. Contrary to our result Krnjaja [22] identified 92.22% of *Fusarium* species as the most common species, followed by the species of the genera *Aspergillus* (80.83%) and Penicillium (48.68%). Alptekin [23] found Penicillium species to be significantly higher than the species of the genera *Aspergillus* and *Fusarium*, in 30 tested samples of maize originating from different localities in Turkey. These variations in contamination levels may be due to differences in climatic and environmental conditions of the study areas. Gong [35] reported that temperature, relative humidity and amount of rainfall may be jointly responsible for the level of contamination of maize grains.

Otsuki [36] also emphasized that factors such as harvesting method, handling, processing, storage and even climate can influence the presence and abundance of aflatoxins producing fungi in food products.

CONCLUSION

A high level of fungal contamination was observed in this study. The most common genera isolated in this study were *Aspergillus, Fusarium*, Rhizopus and Penicillum species which might produce toxin under high humidity and temperature. Maize grains are susceptible to fungal invasion before, during and after harvest. This may be due to improper handling, transportation, inadequate drying and/or poor storage. Fungal infestation may have grave implications on food safety as it makes food unfit for human consumption and complicate public health issues.

RECOMMENDATION

Due to the high level of fungal contamination found in this study, there may be need for the government and other relevant authorities to organize workshops and trainings for the people on the proper method of harvesting, drying, handling, transportation and storage.

Hazard Analysis Critical Control Point (HACCP) should be employed in food processing chain to help reduce fungi infection and subsequent aflatoxin contamination of food commodities. It may also be necessary for the producers and other stake holders

to avoid fungi contamination of their produce as this might constitute huge losses and health hazard. It may be needful for further research to be carried out investigating the best method of preventing and controlling these fungi.

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