# Pathogenicity of Seed-borne Mycobiota of Maize (*Zea mays* (L.) Seeds obtained from Benue State, Nigeria

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***ABSTRACT***

*Reduction of seed viability and germination have been attributed to seed-borne fungi on agricultural seeds. In vitro seed-health studies carried out at the plant Pathology Laboratories of the University of Agriculture, Makurdi, Nigeria, showed the association of maize seeds (Yellow) with C. lunata, B. theobromae, R. stolonifer, A. niger, A. flavus and F. oxysporium. Incidence of 36%, 22.39%, 11.65% were recorded for A. flavus, A. niger, and R. stolonifer, and F. oxysporium respectively with A. flavus being the most occurring organism. It indicated that the organisms caused 5% mean germination failure in the assayed seed. Pathogenicity tests revealed that all the organisms with the exception of R. stolonifer affected the seedlings resulting in varied symptoms on the plants. Controlling these mycobiota of maize seeds could translate to improved crop establishment and productivity.*

***Key Words**: Seed-borne mycobiota, Aspergillus niger, A. flavus, Curvularia lunata, Maize*

## INTRODUCTION

Maize (Zea mays L.) is a ceeal crop belonging to the family Poaceae (Gramineae). Its center of speciation is reported as Meso-America. Today however, the crop is grown in over 100 countries of the world. Maize attains a height of 3 m. the stem is composed of about 20 internodes and the leaf (9 x 120 cm) grows from the internodes. The crop is monoecious with male and female flowers borne on separate terminal inflorescences on the same plant. The kernel bearing ears develop above a few leaves about the mid section of the plant between the stem and the leaf sheath. (Verheye, 2016). Kernel colours may be yellow, white, red, purple or brown (EOLSS, 2016).

Maize is used as fodder, feed, vegetable, and industrial raw material. It is reported as the most important cereal in the world after wheat and rice (Hussain *et al.,* 2013). As food, it forms the staple for 1.2 million people in Africa south of the sahara and the Americas (IITA, 2009); being

consumed as vegetable or processed into several flour-based food forms. In developed economies, it is used primarily as feed and raw materials for pharmaceutical, beverages and confectionery industries. In 2008 in the USA for example, 133.40 million MT, 92 million MT and 8.33 million MT were used for feed, biofuel and human food respectively (USDA, 2016). Maize is rich in vitamins A, C and E, carbohydrate, essential minerals, protein, fibre and calories (IITA, 2009). One hundred grams (100 g) of the cooked grains (Var. Yellow) delivers 1528 KJ of metabolizable energy, protein (9.4 g), fat (4.74 g), carbohydrate (74 g), fibre (7.3 g), Ca (7 mg), Fe (2.1 mg), Mg (127 mg), P (210 mg), K (287 mg), Cu (0.31 mg), Mn (0.49 mg), Se (15.5 µg), B-complex (0.2-3.63 mg), folate (195 µg), vitamin A (214 IU), Vitamin E (0.49 mg), K1 (0.35 µg), unsaturated fatty acids (0.18-0.21 g) and saturated (0.18 g) (USDA, 2016).

Worldwide, 785 million MT of maize grains were produced annually on 158 million ha of farmland, with 42% of this production contributed by the USA and 6.5% by Africa on 29 million ha (IITA, 2009). Annually 8.63 million MT are produced in Nigeria (Ammani *et al.,* 2010). Many of this production is done in the middle and northern belt states (Adamawa, Bauchi, Borno, Yobe, Jigawa, Gombe, Taraba, Plateau, Sokoto, Kebbi, Katsina, Nasarawa, Niger and Zamfara) (Foramfera, 2016) of the country where sunshine is adequate and rainfall moderate (Iken and Amusa, 2004). Grain yields of 4-6 tonnes/ha is reported in northern Nigeria whereas 2-4 tonnes/ha is typical of southern part of the country (ICS-Nigeria, 2016).

However, several factors have been reported to limit the production of this *valuable* cereal. These include low rainfall and insolation amounts, poor soil fertility (Ammani et al., 2012) and biotic pressures from stem and ear borers, parasitic weeds (*Striga hermontica*) and microbial diseases in both field and storage (IITA, 2009). Twenty eight fungal diseases affect the crop at seedling stage, 12 of which are seed-borne (Debnath *et al*., 2012). Seed-borne fungi of the genera *Aspergillus, Fusarium, Curvularia, Bipolaris,* and *Penicillium* have been reported to be associated with maize seeds (Hussain *et al*., 2013). These fungal pathogens affect maize systemically *reducing* its nutrient quality and quantity, causing seed rots, seedling *blight,* germination failure, depressed seedling vigour and poor crop performance (Denath *et al.,* 2012; Hussain *et al*., 2103; Enyiukwu and Ononuju, 2016). In addition, seed-borne mycobiota have been reported also to contaminate maize grains with hazardous metabolites implicated in several forms of allergies, birth defects, cancers and even death in livestock and humans (Enyiukwu *et al*., 2014). Hence this paper evaluated the incidence of fungal biota on maize and the effects of these organisms on the germination and seedling performance of the crop.

## MATERIALS AND METHODS

### Sources of seeds (kernels)

The experiment was conducted in the Pathology Laboratory of the Department of Crop Production of the University of Agriculture, Makurdi. The seeds of yellow maize variety sourced from the Strategic Grain Reserve (SGR) Makurdi, Benue State, Nigeria were used in the study.

### Preparation of culture medium

Thirty nine point five (39.5) grams of dehydrated (synthetic) potato dextrose agar (PDA) (Oxoid™ ThermoScientific Product, England, UK) was dissolved in 1000 ml of sterile distilled

water to which 20mg gentamincin was added (to kill any bacterial cells contaminating the medium), stirred thoroughly with a glass stirrer and then stoppered with a foiled cotton wool. The container with its content was autoclaved at 15 Psi (152cmHg, $120^0$C) for 30 minutes.

**Isolation and identification of seed-borne organisms**

***In vitro* experiment**

Maize (*Zea mays* L.) (Var. Yellow) seeds collected from the Strategic Grain Reserve Makurdi. were sterilized in 70% ethanol for 1 minute and finally washed in several changes of 100 ml of sterile distilled water. Two hundred (200) seeds were plated in Petri dishes containing two layers of moistened Whatman No. 1 filter paper covered and incubated for 7 days at $27^0$C. The seeds were plated 10 seeds per plate, and the plates were laid out in a completely randomized design (CRD) consisting of a single treatment replicated 10 times. The whole experiment was repeated twice. The mycelial growth from the plated tissues was sub-cultured repeatedly to obtain pure cultures of the fungal organisms which were maintained on PDA as prepared above. Records of the number of germinated maize seeds and types of the seed-borne fungi that grew out from the plated seeds and sporulated were taken daily for the 7 days of the incubation per replicate.

The percentage seedling emergence from the maize seeds was assessed based on the formula by Enyiukwu and Ononuju (2016) as:

$$\textbf{\% Seed germination} = \frac{\text{Number of germinated seeds from the plated maize seeds}}{\text{Total number of maize seeds}} \times \frac{100}{1}$$

The colour and colony characteristics of the isolate(s) that grew out of the seeds were observed separately under the microscope. Slides of each of the organisms were prepared, fixed, mounted and examined under a low-high power compound microscope. The morphological characteristics of the conidia and structures of the pathogens were compared and their identity determined with reference to the illustrated genera by Barnette and Hunter (1995).

***In vivo experiment***

Maize (Zea mays L.) seeds surface-sterilized in 0.5% sodium hypochlorite solution for 1 minute and then rinsed in 3 changes of 200 ml of sterile water were sown 3/4kg heat sterilized top soil contained in 20 cm diameter pots. This was later thinned to 2 seedlings/pot. Four days after sprouting, the seedlings were seperately spray-inoculated to run-off with respective spores suspension of the mycobiota (*F. ozysporium, B. theobromae, C. lunata, R, stolonifer, A. niger*, and *A. flavus*) ($10^5$ spores/ml of distilled water), and incubated in humid chambers comprising of transparent lightweight polythene bags for 48 hrs.. The control were similarly set up but sprayed only with sterile distilled water. The potted seedlings were arranged in a randomized complete block design (RCBD) made up of 7 treatments and 5 replicates. The whole experiment was repeated twice and observed for disease initiation, development and symptoms from the sixth day. The influence of the mycobiota on the seedlings of maize was assessed by visual assessment of each seedling for typical disease symptoms (chlorosis, necrosis, root rot, stem rots, wiltting or death) (Awurum, 2014). Records of the symptoms were also taken per treatment per replicate.

The % incidence of the disease on the inoculated maize seedlings was determined as:

$$\% \text{ Incidence} = \text{Number of plants with diseases} \times 100$$

Total number of plants examined 1

**Pathogenecity test** The test fungi in the infected seedlings were re-isolated from the potted diseased maize seedlings, mounted on a slide and re-examined to ensure that their identity conform to the previously isolated and inoculated isolate from maize seeds obtained from the Reserve farm on PDA. The isolates whose identity conforms to the original isolates were confirmed as pathogens and identified whereas those isolates that did not cause any symptoms typical of diseases were regarded as saprophytes.

## STATISTICAL ANALYSIS

All measurements collected from this study were analyzed by simple percentages and analysis of variance (ANOVA) using Genstat 2009 version, at 5% level of significance. Fisher's least significant difference (FLSD) at $p<0.05$ was applied to assess the differences amongst the means.

## RESULTS

The results in Fig 1 indicated that from a total of 200 seeds, 103 seeds (representing 51.50%) were associated with various seed-borne fungi, and 95% germination profile (representing 190 seeds) was recorded from the evaluated seed lot. Also the results of the *in vitro* experiment showed that the *A. niger, A. flavus, F. oxysporium, R. stolonifer, C. lunata,* and *B. theobromae* were the individual mycobiota associated with the seed of maize (Var. Yellow).

The frequency of the individual mycobiota associated with the seeds of maize is presented in Table 1. It indicated that the *A. favus* was most commonly isolated organism from the seeds with a mean frequency of occurrence of 37% followed by 22.3% recorded for *R. stolonifer* and A. *niger* respectively while *C. lunata and B. theobromae* were the least with 3.88% a piece (Table 1).

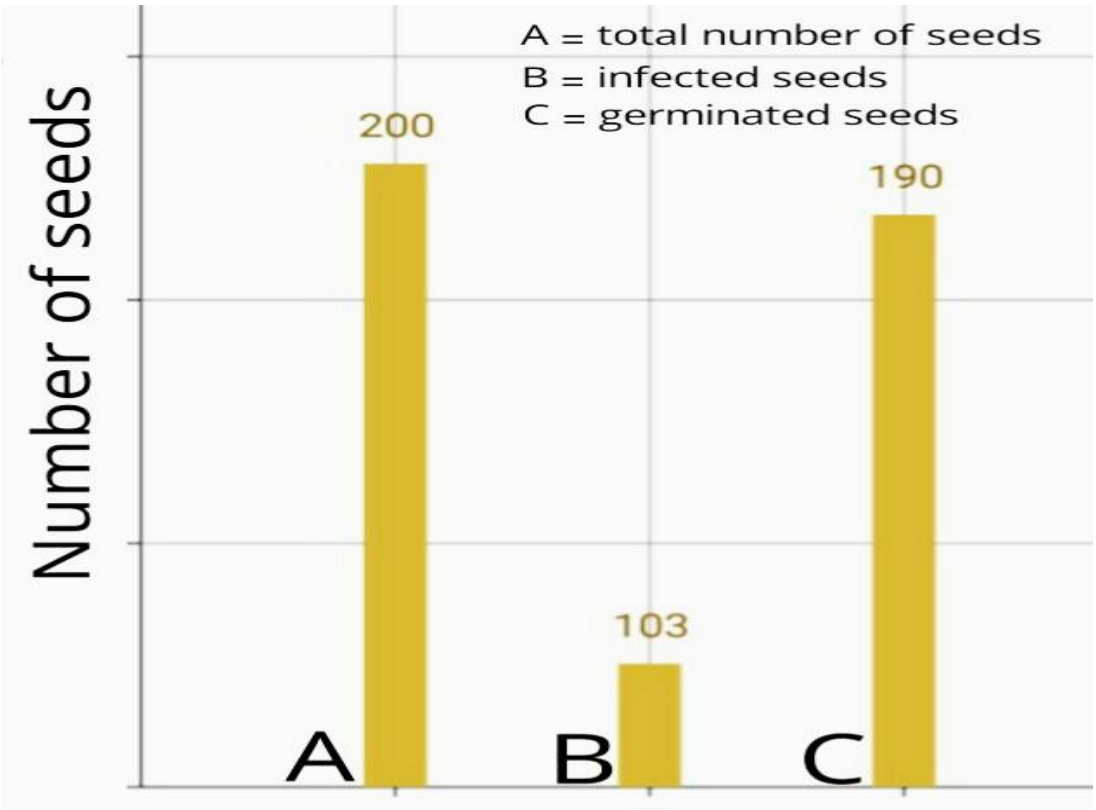


**Fig. 1: Mycobiota isolated from maize (Var. Yellow) seeds obtained from Benue State, Nigeria and their effects on the seed germination.**

**Table 1: Distribution of the individual seed-borne fungi on the maize (Zea mays L.) seeds**

| Organism isolated | Number of seeds infected | % Number of infected seeds |
|---|---|---|
| *Aspergillus flavus* | 37 | 36.00 |
| *Aspergillus niger* | 23 | 22.30 |
| *Fusarium oxysporium* | 12 | 11.65 |
| *Rhizopus stolonifer* | 23 | 22.30 |
| *Curvularia lunata* | 4 | 3.88 |
| *Botrydiplodia theobromae* | 4 | 3.88 |
| LSD (0.05) | - | **2.07** |

Results presented in Table 3 indicated the reaction *of* maize seedlings to the seed-borne mycobiota. It showed that of all the organisms, *F. oxysporium* was the most virulent on inoculated maize seedlings. Ninety percent (90%) of the treated seedlings were affected by the pathogen while 40% actually died from the infection. Affected seedlings presented leaf chlorosis and wilting and rottening of the root system. In *B. theobromae* treated plants, 50% disease incidence and 20% mortality were recorded. The affected seedlings presented strong rottening of the root system. However, chlorotic halo-surrounded lesions were observed on *C. lunata* affected seedlings with incidence and mortality rate of 50% and 20% respectively (Tabl3e 2). These values were significantly ($P \leq 0.05$) different from values obtained from *R. stolonifer, A. niger* and *A. flavus.*

**Table 3: Reaction of the maize seedlings to inoculation with the seed-borne mycobiota**

| Organism | Number of plants inoculated | Number of plants infected | Percentage of plants infected | Symptoms presented by infected plants | % of dead plants |
|---|---|---|---|---|---|
| *A. flavus* | 10 | 3 | 30 | Leaf bligh/chlorosis | 40 |
| *A niger* | 10 | 3 | 30 | Leaf blight/chlorosis | 20 |
| *F oxysporium* | 10 | 9 | 50 | Leaf chlorosis and wilting, root rot | 10 |
| *Rhizopus sp.* | 10 | | Nil | Nil | Nil |
| *B. theobromae* | 10 | 5 | 50 | Strong root rot | 20 |
| *Curvularia sp.* | 10 | 5 | 50 | Haloed leaf spots | 10 |
| Sterile water | 10 | Nil | Nil | Nil | Nil |

## DISCUSSION

Fungal diseases amongst many biotic constraints have been reported to limit profitable maize production in Africa south of the Sahara. Seed-borne mycobiota besides depleting the nutrient contents of agro-produce (Sule *et al*., 2014) are reported to cause seed rot, and to reduce the viability and germination of seeds (Awurum and Enyiukwu, 2013). In this study, 51.50% of the assayed maize seeds were fungi contaminated and showed 5% germination failure. Hussain *et al*. (2013) reported 29.50% germination failure in maize associated with high incidence of mycobiota. This failure in germination was found to increase with increase in fungal load and storage period (Hussain *et al*., 2013; Awurum *et al*., 2014). The finding from this study conforms with theirs. However, the higher germination profile noted in this study may be that the seeds used have not been stored for a long time and consequently the embryo-toxic fungal metabolites were still on the testa of the seeds making their effect to be minimal.

The contamination of seeds of agricultural crops with a wide array of mycobiota has been reported in various literature (Awurum and Enyiukwu, 2013, Awurum and Ucheagwu, 2013). Association of maize seeds with fungi of the genera *Penicillium, Diplodia, Botrytis, Fusarium, Aspergillus, Rhizopus, Curvularia* and *Botrydiplodia* have been variously documented (Kiran *et al.*, 2010; Debnath *et al*., 2012; Hussain *et al*., 2013). Findings from this study where *Fusarium oxysporium, Rhizopus stolonifer, Curvularia lunata, Aspergillus niger, A. flavus* and *botrydiplodia theobromae* are associated with the seeds of maize harmonize with theirs.

The *in vivo* evaluation revealed the reaction of maize seedlings to the various seed-borne mycoflora.It indicated that *C. lunata, B. theobromae,* and *F. oxysporium* were strongly pathogenic to the crop. It showed that A. niger reported previously as agent of maize seedling blight are weak pathogen of the crop. These findings agree with Debnath *et al* (2012) and

Hussain *et al*. (2013) who reported the pathogenicity of these seed-borne fungi to maize seedlings, However, *R. stolonifer* which did not infect the seedlings was only a storage disease.

In conclusion therefore, this work upholds the association of maize seeds with *Fusarium oxysporium, Rhizopus stolonifer, Curvularia lunata, Aspergillus niger, A. flavus* and *botrydiplodia theobromae* . and established that these mycobiota affected the germination of maize seeds. It also indicated that all the fungal organisms with the exception of *R. stolonifer* showed varied levels of pathogenicity to the maize seedlings. Hence, controlling these seed-borne fungi would improve maize seed health and germination which could translate to improved crop establishment and yield.

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