The Mycoflora Of Two Ghanaian Maize (Zea Mays L) Varieties (Abeleehi And Obaatanpa).

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ABSTRACT

The mycoflora of two recently-developed maize (Zea mays L) varieties Abeleechi and Obaatanpa have been studied under varying ambient equilibrium relative humidities ERH's (55, 60, 65, 70, 75, 80, 85, 90, and 95%) representative of the Ghanaian ambient condition. About thirty (30) and twenty-eight (28) species of fungi belonging to the genera *Aspergillus,* , *Clasdosporium, Penicillium, Curvularia, Chaetomium, Emericella, Eurotium, Fusarium, Paecilomyces, Mucor, Neurospora* and *Rhizopus* were isolated from Abeleehi and Obaatanpa varieties respectively at ERH's 55 – 95%. *Aspergillus* species (*Aspergillus candidus, A. effusus, A. fumigatus, A. giganteus, A. niger, A. ochraceus,* (= *A. alutaceus*), *A. sulphureus, A . tamari, A. ustus, A. versicolor, A. wentii*, and *Aspergillus* species) predominated over the others followed by Penicillium (*Penicillium brevi-compactum, P. critinum, P. verrucosum, P. glabrum* and *P. nigricans*). Fungi belonging to the other genera encountered were *Curvularia, Paecilomyces, Chaetomium, Cladosporium, Emericella, Eurotium. Fusarium, Mucor.* The species diversity was influenced by grain variety and the ERH at which the grains were stored. *Aspergillus flavus* was ubiquitous and was encountered in all grains stored at 55 – 95% ERH. *Fusarium moniliforme* was isolated from some grains incubated at 65 – 95% ERH. Xerophillic or xerotolerant fungal species like *Aspergillus fumigatus*, *A. alutaceus* (= *A. ochraceus*), *A. giganteus*, *Paecilomyces carneus*, *P. puntoni*, and *P. varioti*, were isolated at 55 – 65% ERH in both grain varieties.

INTRODUCTION

Maize (*Zea mays* L) is an important cereal grains in Africa ranking as high as rice as a staple food. In Ghana, maize is an important crop cultivated throughout the country with varying degrees of success depending on edaphic and climatic factors.

Being a seasonal crop, especially in West Africa, maize is stored as dry grains and forms an enormous reserve of food.

The microflora of cereal grains is varied and includes moulds, yeasts and bacteria. Generally, bacteria are not significantly involved in the spoilage of dry grain and become a spoilage factor only after extensive deterioration of the grain has occurred and high moisture content exist. (Bullerman, L.B. and Bianchini, A. 2009).

There are more than 150 species of filamentous fungi and yeasts on cereal grains. But the most important of these are the filamentous fungi or moulds. The filamentous fungi that occur on cereal grains are divided into two groups, depending on when they predominate in grain in relation to available moisture in the grain. These groups have been referred to as field fungi and storage fungi. Field fungi invade grain in the field when the grain is high in moisture (i.e. 18 to 30%) and at high relative humidities (90 to 100%). Field fungi include species of Alternaria, *Alternaria, Clasdosporium, Fusarium* and *Helminthosporium.* Storage fungi on the other hand invade grain in storage at lower moisture content (14 to 16%), and lower relative humidities (65 to 90%).These main storage fungi are species of *Eurotium*, *Aspergillus* and *Penicillium*.

The major effects of fungal deterioration of grains include decreased germination, discolouration, development of visible mould growth, musty or sour odour, dry matter loss and nutritional heating, caking and the potential for production of mycotoxins in the grain. (Bullerman, L.B. and Bianchini A. 2009).

To survive in seed/grain, most fungi must be able to survive dehydration, yet there are two ecologically distinct groups of fungi forming a contrast regarding survival and longevity in seed; hydrophilic fungi – those unable to produce resting spores; oospores or those doing so sparsely thus being dependent on a constant humid (≥85% ERH) and xerophillic fungi – those fungi which are characteristically capable of producing xerotolerant propagules, often abundant such as chlamydospores, including dormant mycelium, sclerotia and microsclerotia (Neergaard, 1983). Most xerotolerant conidia become quiescent if the Environmental Relative Humidity remains too low for germination usually 80% ERH.

In an attempt by man, to cultivate new species and varieties of crops suited to his needs, the Crops Research Institute of the Council for Scientific and Industrial Research (CSIR) of Ghana has through the Grains and Legumes Improvement Programme, developed high Lysine content maize grains including Abeleehi and Obaatanpa, Okomasa, Dobidi, to name but a few which are being sold to the local farmers. However, there is hardly any information on the mycoflora associated with these grains which have to be stored for prolonged periods as seed grains for the

next planting season. This paper is an attempt to document the mycoflora associated with Abeleehi and Obaatanpa and show their pathological effect on the crop under greenhouse and field conditions.

MATERIALS AND METHODS

i). MATERIALS

The maize varieties used Abeleehi and Obaatanpa were purchased from Aglow Seed Company, Accra ,Ghana.

ii). GENERAL METHODS

Maize samples of Abeleehi and Obaatanpa were kept at 55, 60, 65, 70, 75, 80, 85, and 95% Equilibrium Relative Humidity (ERH) provided by glycerol; water mixtures and at temperature of 28 - 31°C for 36 days.

Table 1: Maintenance of Environmental Relative Humidity with Glycerol: water mixtures

% ERH	Vol. of glycerol (ml)	Vol. of water (ml)
20	94	6
55	75	25
65	68	32
75	58	42
85	45	55
95	22	78

Direct-Plating Method

The maize grains were surface-sterilized by washing in Milton's reagent (1% sodium hypochlorite + 16.5% sodium chloride) for 5min and then rinsed with three changes of sterile water.Sodium hypochlorite treatment was used with the aim of reducing or removing completely external saprophytes which compete with pathogens. Ten surface-sterilized grains were placed on wither Sabourand Dextrose Agar (Oxoid CM 41) Dichloran Glycerol Agar DG 18 (Oxoid CM 727) in Petri plates without further treatment. Plates were incubated until fungi grew.

There were 25 replicates for each variety.

Serial-Dilution Method

A 10g sample of the grains was weighed and transferred aseptically into 100ml 0.1% Peptone in 250ml Erlenmeyer flasks and then shaken in Gallenkamp Model Orbital shaker at 140 rev/min for 30 mins. From this stock suspension serial dilution method was employed up to 1:10v/v.

Spores were raised in Sabourand's Agar(Oxoid CM 41) or Oxytetracycline Glucose Yeast Extract Agar(Oxoid CM 545). The objective of using two media was to recover a wider range of fungal species from the incubated grainS

The plates were incubated at 28 - 31°C until fungi grew (7-14 days)

Identification of Fungi

Fungi encountered in these investigations were identified by their colour, culture and morphological characteristics using the conventional identification manuals of fungi by;

Barnett and Hunter (1972), Ellis and Ellis (1988). Funder (1953), Gilman (1957), Samson and Reenen – Hoeskstra (1988) and Smith (1960).

Where necessary, the identification of the fungi were confirmed by G.T Odamtten professor of mycology, Botany Department, University of Ghana, Legon.

Results and Discussion

Results

The list of seed-borne fungi isolated from the two maize varieties is presented in Table 2 below:

Table 2:

List of seed-borne Fungi isolated from maize varieties Abeleehi and Obaatanpa incubated at 55 – 95% Equilibrium Relative Humidity for 30 days at 28 - 31°C.

Aspergillus candidus Link ex. Fr [1,2]	*P. expansum* Link [1,2]
A. effuses Tiradoschi [2]	*P. funiculosumm* Thom [2]
A. flavus Link ex Fr [1,2]	*P. glabrum* (Wehmer) Westling [1,2]
A. fumigatus Fresenius [1,2]	*P. nigaricans* Bainier [1]
A. giganteus Wehmer[1]	*Penicillium* index [1,2]
A. niger Van Tieghem [1,2]	*Paecilomyces carneus* (Duche *et* heim) A.N Brown G. Smith [1,2]
A. sulphureus [1] (Fresenius) Thom and Church	*P. puntonii* (Vuillemin) Nannizz [1,2]
A. tamari Kita [1,2]	*P. varioti* Bainier [1,2]
A. *terreus* Thom Geerlings **[1,2]**	*Chaetomium globosum* Kunze Fries [2]
A. ustus Bainier Thom and Church [1]	*Cladosporium herbarum* (Person Fries) Link [1,2]
A. versicolor (Vuillemin) Tirabaschi [2]	*Curvularia lunata* Boedji [1,2]
A. wenti Wehmer [1]	*Emericella nidulans* (Eidam) Vuill [1]
Aspergillus indet [2]	*Eurotium* sp. [1,2]
Penicillium brevi-compactum Dierckx [1,2]	*Fusarium moniliforme* Sheldon [1,2]
P. citrium Thom [1,2]	*Mucor haemalis* Welmer f. hiemalis [1]
P. verrucosum Dierckx [1,2]	*Rhizopus oryzae* Went and Pri [1,2]
P. digitatum Sacc [1,2]	*Scopulariopsis brevicaulis* (Sacc) Bain [2]
	Neurospora sitophila Shear and Dodge [1,2]
1 – Abeleehi variety	

2 – Obaatanpa Variety	

Discussion

Seed-borne fungi isolated from Abeleehi and Obatanpa are being recorded for the first time in these varieties in Ghana. About thirty different seed-borne fungal species were isolated from Abeleehi and twenty-eight from Obaatanpa varieties at the varying equilibrium relative humidities (55, 60, 65, 70, 75, 80, 85, 90 and 95%) at which the grains were stored.

Aspergillus species (*A. candidus, A. effuses, A. fumigatus, A. niger, A. alutaceus* (= *A.* ochraceus) *A. sulphureus, A. tamari, A. ustus, A. versicolor, A. wentii,* and *Aspergillus* sp. 1) predominated over other species encountered followed by *Penicillium* (*P. brevi-compactum, P. citrinum, P. glabrum, P. nigricans* and *Penicillium* sp.) Fungi of other genera (*Curvularia, Paecilomyces, Chaetomium, Cladosporium, Emericella, Eurotium, Fusarium, Mucor, Neurospora* and *Rhizopus* were also isolated.

The species diversity was influenced by grain variety and the ERH at which they were incubated. *A. flavus* was ubiquitous and was isolated from Abeleehi and Obaatanpa stored at ERH 55 – 95%, *Fusarium moniliforme* was encountered at ERH 65 – 95%. Xerophilic species like *A. fumigatus, A. giganteus, A. alutaceus,* (=*A. ochraceus*), *Paecilomyces carneus, P. puntoni* and *P. varioti* were isolated at ERH's 55 – 65% in both grain varieties.

Paecilomyces varioti produces patulin (Frisvad, 1988) but the nature of the mycotoxins from *P. carneus, P. puntoni*, have not clearly elucidated. *Fusarium moniliforme* is one of the most prevalent fungi associated with maize, a basic human and livestock dietary staple. (Marasas, *et al* 1984) Experimental studies in South Africa (Marasas, *et al*, 1984) and in China (Li and Cheng, 1984. Lin and Tang, 1980, Yang, 1980) have shown that cultures of *F. moniliforme* on maize and maize products can cause cancer in rats; *hepatocaricinogens* produce by one strain can survive drying of the maize at 45 - 50°C for 24 hours. (Marasas *et al,* 1984). At least two classes of mutagens are formed by *F. moniliforme* namely Fusarin C (Wiebe and Bjeldanes, 1981; Gelderblom *et al* 1982; Scott *et al*, 1986). Fusaric acid is a phytoxic compound (Marasas *et al*, 1984 a,b). Fusariocins A and C (Arai and Ito, 1970) have also been isolated from the metabolites of *F. moniliforme*. Thus, the danger to human and animal of *F. moniliforme*-infected maize is self evident. Furthermore, it is well established that *F. moniliforme* can be internally seed-borne in symptomless apparently healthy maize kernel, (Foley, 1962; Marasas *et al* 1979; Thomas and

Buddenhagen, 1980). *Aspergillus ochraceus* (= *A. alutaceus)* forms *ochratoxin* A in maize (Frisvad, 1988) as well as other mycotoxins such as emodin, kojic acid, neospergillic acids, penicillic acid, secalonic acid *A. Viomellenin* and *Xanthomegnin* (Frisvad, 1988). Many other fungal flora encountered in Abeleehi and Obaatanpa in this investigation produce mycotoxins in foods and may have serious public health implication if ingested by the consumers. It is therefore, imperative that future investigations are carried out to quantify production of potent mycotoxins such as *aflatoxins* (*A. flavus*) cyclopiazonic acid (*A. tamari) ochratoxin* and Penicillic acid (*A. alutaceus* = *A. ochraceus*), patulin (*Paecilomyces* species) to mention but a few in maize stored for prolonged period for human and animal consumption.

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