2.

Review of Literature

2.1 Fungi Associated with Cereal Grain

The fungal flora associated with grain has traditionally been considered to fall into two categories: field fungi and storage fungi (CHRISTENSEN, 1957). This classification has widely been applied to toxigenic fungi as well. PLACINTA *et al.* (1999) consider field fungi to be plant pathogenic and storage fungi to be saprophytic, but recognize the presence of intermediate forms of toxigenic fungi. A more specific classification was given by MILLER (1995) who recognized four types of toxigenic fungi: a) plant pathogenic fungi such as *Fusarium graminearum*, b) fungi such as *F. moniliforme* and *Aspergillus flavus* that grow and produce mycotoxins on senescent plants or on plants whose resistance is weakened due to stress factors, c) fungi including *A. flavus* that initially colonize the plant and predispose the commodity to mycotoxin contamination after harvest, and d) fungi that are found in the soil or in decaying plant material and infest the developing kernels in the field and later proliferate in storage if suitable conditions prevail (e.g., *Penicillium verrucosum* and *A. ochraceus*).

2.1.1 Field Fungi

A wide range of different fungi invade the grain before or at harvest. Field fungi grow on seeds with more than 20% moisture content. The most important species are from the genera *Alternaria*, *Cladosporium*, *Fusarium* and *Drechslera* (CHRISTENSEN, 1979; TROJANOWSKA, 1991; SCUDAMORE, 1993). *Fusarium* spp. cause head blight which affects the ears of the cereal grain while *Alternaria* and *Cladosporium* cause a disease complex known as sooty mold (SCUDAMORE, 1993). In sorghum grain, *Fusarium moniliforme* and *Curvularia lunata* are of worldwide importance among the fungi that infect

spikelet tissues during early stages of development (WILLIAMS and RAO, 1981; BANDYOPADHYAY, 1986). In addition, *Fusarium semitectum* and *Phoma sorghina* infect sorghum grain before harvest and could also become important (FORBES *et al.*, 1992).

Fusarium species are the most important for pre-harvest mycotoxin production in cereals; *F. culmorum*, *F. graminearum*, *F. poae*, *F. tricinctum*, *F. sporotrichioides* and *F. avenaceum* have all been recorded as mycotoxin producers *in vitro*, and many of their mycotoxins have been shown to occur in cereals (SCUDAMORE, 1993). Fusaria can be regarded as psychrophilic (NORTHOLT *et al.*, 1995), but it is important to appreciate that some species such as *F. moniliforme* are of considerable importance in the tropics (MOSS, 1991).

2.1.2 Storage Fungi

After harvest, storage fungi (species of fungi which proliferate at lower seed moisture levels) tend to replace the fungi found in the growing crop. A great variety of fungi has been isolated from stored grain (e.g., FLANNIGAN, 1969; 1970) although the role of most of these fungi in grain deterioration is not always well understood.

The major storage fungi that are associated with grain comprise about a dozen species of *Aspergillus* and several species of *Penicillium* (CHRISTENSEN and KAUFMANN, 1969). The most important mycotoxin producing fungi after harvest also belong to these genera. Under favorable conditions, certain strains of Fusaria (which normally are field fungi) may proliferate in storage and produce mycotoxins. In general, Penicillia are important in the temperate climates whereas Aspergilli predominate in the tropics (NORTHOLT *et al.*, 1995).

2.2 Mycotoxins

Fungi produce a wide array of secondary metabolites, many of which may be extremely toxic to humans and animals. These toxic, small molecular weight compounds have been named mycotoxins. Mycotoxins are usually produced after a phase of balanced growth and occur in the mycelium of filamentous fungi as well as in the spores of these organisms (D'MELLO and MACDONALD, 1997). Because of the remarkable propensity of toxigenic strains of fungi to produce mycotoxins, invasion or colonization of food and feed products by such fungi usually leads to contamination of the products with mycotoxins. The adverse health effects in humans and animals elicited by ingestion of food or feed that contains mycotoxins are collectively called mycotoxicoses.

2.2.1 Agriculturally Important Mycotoxins

More than 200 toxic fungal metabolites have been identified from *in vitro* cultures of fungi and were characterized chemically and toxicologically (COLE and COX, 1981). Out of these, about 20 have been found to occur in foodstuffs, constituting the significant mycotoxins in food and feed (POHLAND and WOOD, 1987). MILLER (1995) evaluated extensive analytical results on the occurrence of mycotoxins and information on the distribution of fungi in staple crops; he concluded that there are only five agriculturally important mycotoxins, namely, aflatoxins, deoxynivalenol (replaced in some areas by nivalenol), fumonisins, ochratoxin A and zearalenone. In the following sections information pertaining to these toxins are presented.

Mycotoxins are as varied as the fungal species that produce them, and they constitute a group of compounds of diverse chemistry and structure, some of which are presented in Fig. 1 (adapted from Anon., 1993a).

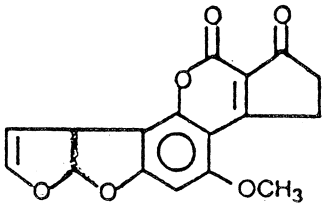
2.2.2 Mycotoxin-Producing Fungi

Particular mycotoxins are produced by one or a limited number of fungal species only, and not all isolates of a given toxigenic species can produce its specific toxin. Hence, only *Aspergillus flavus*, *A. parasiticus* and *A. nomius* produce aflatoxins (KURTZMAN *et al.*, 1987; FRISVAD and THRANE, 1995), and the ability to produce aflatoxins is most consistent in the latter two species (FRISVAD and THRANE, 1995).

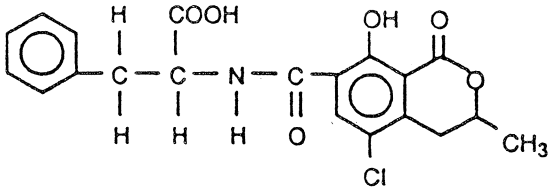
It is now widely accepted that ochratoxin A is produced by only one species of *Penicillium*, namely *P. verrucosum* (FRISVAD and FILTENBORG, 1989) and by a number of species of the *A. ochraceus* group, including, *A. alliaceus, A. melleus, A. ochraceus, A. sclerotiorum* and *A. sulphureus* (CIEGLER, 1972).

Strains of *Fusarium cerealis* (=*F. crookwellense*), *F. culmorum* and *F. graminearum* produce deoxynivalenol (MARASSAS *et al.*, 1984; THRANE, 1989; FRISVAD and THRANE, 1995). Most naturally occurring nivalenol is a product of strains of these three species of *Fusarium* (MILLER *et al.*, 1991; FRISVAD and THRANE, 1995). Nivalenol-producing isolates of *F. graminearum* appear to occur primarily in Japan, Australia and New Zealand

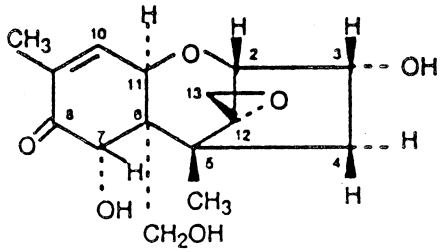
(BLANEY, 1991), but they have also been reported from Italy (LOGRIECO *et al.*, 1988). Isolates of *F. poae* also produce nivalenol (SALAS *et al.*, 1999).



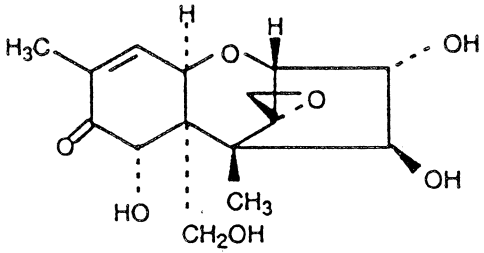
Aflatoxin B_1



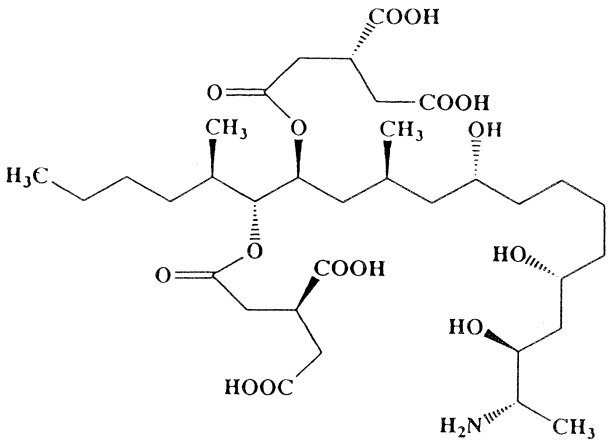
Ochratoxin A



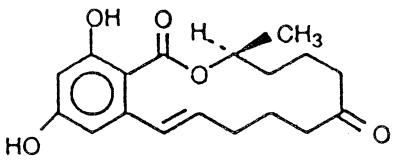
Deoxynivalenol



Nivalenol



Fumonisin B_1



Zearalenone

Figure 1. Chemical structures of agriculturally important mycotoxins