Chapter 1
Chapter I

INTRODUCTION

Foodstuff whether of plant or animal origin is often subject to deterioration. Of the different agencies causing decay, fungi, a group of thallophytes are largely responsible for deterioration of cereals, fruits and vegetables.

In the tropical climate of India, harvesting practices post harvest storage practices, high temperature, high moisture level during monsoon seasons, unusual rains and sudden floods damaging standing crops and stored food grains are the areas in which fungal contamination, invasion and elaboration of mycotoxin is of considerable importance. Paddy, one of the major staples in many parts, is often harvested with high moisture content. Inadequate drying of paddy after parboiling is an important factor contributing to disease onset. It is also known that most of the diseases induced production of toxins in the tissues causing toxaemia.

The pioneer workers to recognize that fungi are involved in deterioration of stored grains were Ramstad and Geddes (1943). Kelly (1940) also observed that the moisture content of the grain could be reduced by ventilation with subsequent fall in infection rate.

Delprado and Christensen (1952) stated if a given seed lot has a very high count of a species of fungus known to invade the seed and to cause loss of viability in storage,
there is presumptive evidence that the seed is deteriorating or has already deteriorated. Since storage mould increase rapidly in high moisture and under conditions where damage and heat damage often occur one would expect a direct relation between increase in storage moulds and increase in 'damage' and 'heat damage'.

Sorger-Domenigg et al (1955) working with wheat stated that a combination of tests involving moisture control, number of kinds of moulds present, viability and fat acidity should serve to predict storage behaviour and extent of actual damage. The moisture content should indicate whether there is present or future danger, mould tests whether invasion of the seed already has occurred, viability whether incipient deterioration has developed and fat acidity should give some measure of the actual damage which already has occurred.

Quasem and Christensen (1958) stored corn samples of 17% to 18% moisture content for two years at 15°C free of storage fungi as well as incubated with storage fungi. The samples free of storage fungi retained a germination percentage of 96% whereas in the samples inoculated with storage fungi, the germination percentage was as low as zero. Koehler (1957) noted that pericarp injuries of the seed facilitated invasion of the seed by fungi.

Christensen and Kaufmann (1965) found out that the lower limit of moisture content that permits invasion of starchy cereal seeds by A. flavus group is 17.5% to 18.0% on wet weight basis. Panasenko (1967) stated that A. niger
requires relative humidity (RH) range of 88% to 100%, Botrytis cineria requires 92% to 100% and Rhizopus stolonifer requires a range of 92% to 100%. It has been reaffirmed by him that in the growth of fungi the question of relative humidity cannot be set apart.

Mehta (1971) stressed the importance of relative humidity (RH) in the deterioration of foodstuff. Boller and Schroeder (1974) reported that storage fungi like A. parasiticus require RH of 70%, 75% and 85% to grow. It is an important point as stated by Christensen and Mirocha (1976) that A. flavus when grows mostly in stored products with moisture content in equilibrium with 75% RH, the aflatoxin hazard is much greater than that of its lower limit of growth in a moisture content in equilibrium with 85% RH.

Carter and Young (1950) and Christensen (1955) found that invasion of stored seeds by various species of the genus Aspergillus is now known to be a primary cause of or major contributing factor to the development of germ damage or sick wheat in stored grain. Aspergillus restrictus first reported in stored grain by Tuite and Christensen (1955) has since been found to be a common cause of deterioration in all kinds of stored grain and seeds which have been investigated. Tuite and Christensen (1957) described that invasion of stored wheat by species of Aspergillus is known to result in decrease in germination percentage and in the development of brown to black germs and loss of grain weight due probably to the evaporation of alcohol from the grains during storage. This finding was
confirmed by Iwaski et al (1967). Delprado and Christensen (1952), Tuite and Christensen (1957), Kotheimer and Christensen (1961) and Christensen and Lopez (1963) have observed that under usual conditions of food and foodgrain, Fusarium may die within a few months; but Helminthosporium may not be recoverable from more than a small percentage of seeds stored for a year. It is also observed under certain conditions that the storage fungi cause loss of germination of seeds (Christensen, 1964).

Although it has been stated by Christensen and Kaufmann (1965) that Alternaria, Cladosporium, Fusarium, Helminthosporium, Pilularia to be field fungi, yet species of Fusarium and Helminthosporium, in particular, play an important role in the deterioration of stored cereal foodstuffs. Tikko et al (1973) found that deterioration of stored grain was due to high activity of enzymes.

The present status of work has been gradually diverted towards the toxic effects of consuming contaminated food from the storage.

It may be of interest to determine if toxins elaborated by the pathogens or induced to be formed as reaction products are responsible for the toxic effects on the tissues. The fungi responsible for causing the disease in swine were isolated but the toxic compound was not studied. The oestrogenic syndrome in swine detected in late 1820 in U.S.A. was reported to be due to consumption of mouldy feed. In 1900, some Japanese workers suspected that people suffering from beri-beri may be due to consumption of mouldy food.
During 1930-33 in the Ukraine, corn was so damaged that it resulted in mass disease and death of livestock while being fed on it (Panasenko, 1964). In late 1930's and early 40's, reports of widespread outbreak of mycotoxicoses in farm animals occurred due to feeding of over-wintered millet invaded by *Fusarium tricinctum*. Among different types of storage fungi such as *A. flavus*, *A. niger*, *A. fumigatus*, *A. candidus*, *Alternaria* sps., *Curvularia* sps., *Cunninghamella* sps., *Penicillium notatum*, *P. islandicum*, *P. frequentus*, *Helminthosporium* sps., *Rhizopus* sps., toxins are produced by only a few types.

Though many papers on the problem are published, the information of mycotoxicoses did not filter out of the Western world until 1960, when a sudden outbreak of mycotoxicoses occurred in Turkey taking 100,000 lives in England. Blount (1961) defined it as Turkey X disease. Since then over 2,000 papers have been published on the subject of mycotoxins.

The syndrome was soon attributed to toxic substances in animal feeds giving rise to symptoms in the cattle similar to those of ragwort poisoning. Systematic examinations of the various feeds involved revealed an active principle from the toxic peanut meals of Brazilian origin which could produce death and pathological symptoms in ducklings (Allcroft et al 1961, Sargeant et al 1961).

Subsequently many samples of feedstuff and peanut meals were tested for toxicity with the result that some samples
from at least fourteen peanut producing countries were found to be contaminated with the toxic agent arising from the fungal organisms which cause death (Allcroft and Carnagham, 1963).

Tyagi (1964) working on oat seedlings found production of toxin by *H. victorae*. Vidyasekharan (1977) worked with toxin produced by *H. nodulosum* and found inhibition of root elongation as well as leafspot in ragi.

The growth of *Fusarium roseum* Link on barley and rice has been associated with toxicity syndromes in swine and man (Kinosita and Shikata, 1965). Wogan (1969) stated that although aflatoxin was produced on various substrates, yields were poor on soyabeans, maximal on rice and intermediate on other commodities. He further worked out and found that maximum production of toxin from *A. flavus* will be reached in 10 days or less after which it may increase again.

The limiting factor of RH for aflatoxin produced by *A. flavus* was reported to be 85% for 21 days at 30°C (Diener and Davis, 1967).

Citrinin is produced by some species of *Aspergillus* which infect stored maize. It is not a carcinogen but Penicillic acid, a metabolite of *Penicillium puber-ulum* is a carcinogen. Patulin is a carcinogen produced by *Penicillium* and *Aspergillus* (Ciegler et al 1971).

There are many other fungi which are contaminants but *Aspergillus flavus* is known to produce aflatoxin B, which
is proved to be injurious to the liver in all experimental animals including human beings to a serious extent. Aflatoxin was found in one or more specimens out of 22 or 23 autopsies of children who died of acute encephalopathy and fatty degeneration of viscera in Thailand (Shank et al 1971).

Zearalenone is a fungal metabolite of Fusarium. The disease caused due to eating of mouldy foodstuffs invaded by Fusarium causes estrogenic syndrome in swine, general atrophy of the ovaries, infertility and reduced litter size; males undergo feminizing effect of the testes and enlargement of the mammary glands, besides diarrhoea, emesis, haemorrhages, refusal of feed and loss of weight (Smalley et al 1970, Morasses et al 1972 and Hsu et al 1972).

Penicillium virdicatum and Aspergillus ochraceous are known to produce ochratoxin. Ochratoxin causes tubular necrosis of the kidney, mild degeneration of liver and enteritis of small intestine but it is non-carcinogenic in rat (Purchase and Van der Watt 1971). Hald and Krogh (1973) investigating the organs and meat from pigs suffering from nephropathy detected ochratoxin A. They concluded that ochratoxin A may be transmitted through animals to the human food and may pose a public health problem.

It has been stated by Edds (1973) that occurrence of mycotoxicoses is associated with feeding field harvested grains; and feeding of concentrated rations stored under conditions of moisture, temperature and aeration favourable to the toxigenic fungi.
'Toxic Yellowed Rice' is a most notable syndrome. *Penicillium citriviridae*, *P. citrinubo* and *P. islandicum* cause the yellowing of rice. Citreoviridin being a highly toxic metabolite from these causes ascending type of paralysis in man with respiratory and circulatory disturbances, beri-beri, multiple neuritis, general weakness, mental deterioration and heart failure.

The humid climate of Assam is highly favourable for the development of fungi in any kind of substrate.

The consumption of the stored foodstuff in mouldy condition causes great hazard not only to poultry and domestic animals but is often found to cause hepatic carcinoma and cirrhosis on human beings.

The above findings indicate the urgent need to study the role of fungal toxins in food and the steps to be taken in the conditions of storage to minimise contamination and spoilage of foodstuff by fungal organisms.

The aim of the present investigation is therefore to:

1. isolate the fungal species normally present in field, storage bins, grains, seeds and other foodstuffs;
2. study the conditions of their growth and spoilage of foodstuff, viz., moisture content of the food, RH of the storage atmosphere, temperature, etc.;
3. study the process of degradation and changes in the
foodstuff;

(4) investigate the effect of infected foodstuff on animals and birds;

(5) isolate and identify the toxic principles which produce disease syndrome;

(6) review control measures.