CHAPTER I

GENERAL INTRODUCTION
Mycotoxicology deals with the diseases in man and animals caused by or resulting from ingestion of foods made toxic by metabolic products of fungi. The poisoning of food and feed is one of the most important harmful activities of fungi. Although the associations of toxic substances with plants and plant products used for human consumption has been made since biblical times but the role of fungi in food spoilage was recognised only in the later half of the 19th Century. Alsberg and Black (1913) cited many papers of Italian workers from the early 1870's on the possible role of fungi in the spoilage of imported corn. Similar reports on the toxicity of certain other products and their impact on some animals have also been made by some workers in the first half of twentieth century, these includes the toxicity of Brazil nuts by Kuhl (1910), poisoning due to mould on corn by Lagenhausen (1928), Fusarium toxicity and digestive disturbance in Swine by Christensen and Kernkamp (1936) while on the other side reports were made by many workers to find out the associations of micro-organisms and food grains (Black and Alsberg, 1910; Morgenthaler, 1918; Geilinger, 1921; Kent-Jones and Amos, 1930).
A number of reports on the toxicity of animal feed have appeared in the fifth and sixth decade of the present century (Raistrick et al., 1943; Ambrose and DeEds, 1946; Korneev, 1948; Bollog, 1949; Morton et al., 1949; Burnside et al., 1957; Newberne et al., 1955; Forgacs et al., 1958; Clare, 1959). This indicates that during 1950s mouldy feed toxicoses was recognised as a serious livestock problem that took several clinically distinct forms. Some of these early outbreaks of disease among farm animals might well have been caused by aflatoxin(s) but, of course, were never so defined (Newberne et al., 1955; Burnside et al., 1957).

After the deaths of 100,000 young turkeys due to a mysterious disease ('Turkey X disease') in 1960 and deaths of some 14,000 ducklings a joint paper by Allcroft and Carnaghan of the Central Veterinary Laboratory, Weybridge, and Sargeant and O'Kelly of the Tropical Products Institute, London, heralded the aflatoxin era in 1961 (Allcroft et al., 1961). In the same year a number of other workers have published their papers based on the identification of groundnut toxins (Carnaghan and Sargeant, 1961; Loosemore and Markson, 1961; Sargeant et al., 1961a, b, c; Loosemore and Harding, 1961; Asplin and Carnaghan, 1961). Since then, a number of review articles and papers have appeared on mould produced toxins and their toxicity in various animals.
One of the first reviews on mycotoxins was published by Forgacs and Carll in 1962, which includes informations from a large number of paper published in USSR. Other early reviews on mycotoxins and their possible role in causing mycotoxicoses have been presented by Brook and White (1966), Borker et al. (1966), Hesseltine (1969), Herzberg (1970), Ciegler et al. (1971), Kadis et al. (1971), Mirocha and Christensen (1974), Purchase (1974), Rodricks (1976), Wyllie and Morehouse (1977, 1978).

As stated by Mirocha and Christensen (1974), "The following mycotoxins have been found in nature: aflatoxins, zearalenone, trichothecone, citrinin, ergot alkaloids, ochratoxin, slaframine, sporodesmin, penicillic acid, patulin and sterigmatocystin. Of these, the first eight have been implicated in some cases of natural toxicity".

The principal groups of fungi that produce mycotoxins are the species of three genera, i.e., Aspergillus, Fusarium and Penicillium. Species of Aspergillus incriminated in mycotoxicoses, and are known to synthesize mycotoxins on a variety of agriculture produce, are Aspergillus candidus, A. clavatus, A. fischeri, A. flavus, A. fumigatus, A. nidulans, A. ochraceus, A. parasiticus, A. sydowi, A. terreus and A. versicolor. While, some other Aspergilli belonging to Aspergillus glaucus, A. niger and A. restrictus groups are of uncertain mycotoxic
importance (Tuite, 1977). The toxigenic species of genus *Penicillium* include *P. citro-viride*, *P. citrinum*, *P. purpurogenum*, *P. rubrum*, *P. islandicum*, *P. rugulosum*, *P. oxalicum*, *P. expansum*, *P. cyclopium*, *P. martensii*, *P. urticae*, *P. viridicatum*, and some others (Mislivec, 1977). The toxic *Fusarium* species were isolated from various samples of fresh, stored and overwintered cereals, vegetable crops, fruits, soil and from feed grains consumed by man which were implicated in death due to alimentary toxic aleukia (Joffe, 1977). Most of these toxic *Fusarium* belong to section - *Arachnites* Wr., section - *Sporotrichiella* Wr.em. Joffe, section - *Roseum* Wr., section - *Liseola* Wr., section - *Gibbosum* Wr.em. Joffe, section - *Discolor* Wr., section - *Martella*. Most of the species of genus *Aspergillus*, listed above, are well known storage fungi. According to Christensen and Kaufmann (1969) storage fungi are those which grow on stored products, they generally do not invade grain in standing crop or atleast prior to harvest. In contrast to *Penicillium* species, those are known as storage mould, most storage Aspergilli are able to grow without free water or on media with high osmotic pressure.

The losses which occur in stored agriculture produce have been classified into seven grades by Christensen (1978). Amongst these, mycotoxin production is most important from the point of view of their impact
on consumers health. The major toxin which have been implicated in various health hazards are aflatoxins, ochratoxins, zearalenone and trichothecene. Amongst various aflatoxins and their derivatives, Aflatoxin B₁ is carcinogenic, teratogenic and mutagenic, and because of these properties, it is considered to be of prime importance in public and animal health. *Aspergillus parasiticus* is usually found infecting peanuts and produces B₁, B₂, G₁ and G₂. In contrast, *A. flavus* is usually found infecting maize and commonly produces B₁ and G₂ to the exclusion of other derivatives. Aflatoxicol is formed by *A. flavus* and considered dangerous because it acts as a reservoir for aflatoxin B₁ (Mirocha and Christensen, 1976). Other members of aflatoxin group such as M₁ and M₂, P₁, B₂a and G₂a, Q₁ and H₁ are the products of metabolism of B₁ by animals and many of these are non toxic.

Ochratoxin(s) are produced by several individual species of *Aspergillus ochraceus* group and *Penicillium viridicatum*. Ochratoxin A was first found by Van der Merwe *et al.* (1965) in South Africa. They later found the other ochratoxins namely ochratoxin B and C and reported them to be very toxic to ducklings. In general, the main target organs of the ochratoxins are the liver and kidney; it causes tubular necrosis of kidney, mild degeneration of the liver and enteritis of the small
intestine (Krogh, 1974). Ochratoxin has also been implicated in a disease of human beings called Balkan Nephropathy (Austwick, 1975).

Species of Fusarium are wide spread in nature and are found both as a saprophyte and as a parasite in various plant parts. Different species of this genus cause stem and cobrots of corn (maize), head blight of wheat, oats and barley (Mirocha and Christensen, 1976), so that in many regions of the world, consumption of Fusarium infected grains or forage is almost inevitable. Zearalenone is produced by the species of Fusarium and causes the esterogenic syndrome in swine (Kurtz and Mirocha, 1978); toxicosis in poultry (Joffe, 1978; Kurmanov, 1978), in horses (Bridges, 1978) and in cattle and sheep (Kurmanov, 1978). Trichothecone toxins (deoxynivalenol, diacetoxyscirpenol, T-2 toxin) are biologically active secondary metabolites produced by Fusarium tricinctum, F. roseum, F. oxysporum, F. solani and others. The toxins produced by Fusarium when ingested orally are causing diarrhea and rectal hemorrhage. Necrotic lesions may develop in the mouth parts. The mucosal epithelium of the stomach and small intestine erodes developing into a severe case of gastroenteritis which may result in death. The cells of the bone marrow, lymph node and intestine may undergo pathological degeneration (Mirocha and Christensen, 1976).
The literature indicates that the mycotoxins are the great threat to farm animals, birds and certain other living beings. In addition to these a number of reports have also appeared on the impact of mycotoxins on human health (Keen and Martin, 1971; Shank et al., 1972; Peers and Linsell, 1973; Rensburg et al., 1974; Krishnamachari et al., 1975 a and b, 1977). These reports indicate that aflatoxin ingestion may bring about liver cancer in human beings. Generally it is found that males are more susceptible to aflatoxicosis, however, this requires further investigations by using a wide variety of test animals with diversified characters (Bilgrami, 1983).

The toxigenic potential of fungal strains is largely governed by the ecological factors such as temperature and humidity which determine natural contamination of mycotoxins (Sanders et al., 1968; Nagarajan et al., 1973; Gupta and Venkit Subramanian, 1975; Moreau, 1979).

A review of paper published on various outbreaks which occurred in different parts of the world, and the attempts which have been made for the identification of the cause of various sufferings in many mammalian, avian and fish species due to ingestion of toxic food and feed was carried out by Wyllie and Morehouse (1977, 1978). The literature indicates that very few reports were made
by Indian workers on this important aspect till the compilation of above reviews, though several deaths of poor tribes have occurred due to consumption of mouldy grains in some parts of India (Krishnamachari et al., 1975a and b).

In recent years considerable awareness has been shown by the Indian scientist on mycotoxin problem and their research have provided valuable piece of information regarding frequency of toxigenic fungal strains in some parts of our country. They also demonstrated toxigenic potentials of fungal contaminants recorded by them on a wide range of agriculture produce including cereals (Mishra, 1977; Bilgrami et al., 1980a, 1981; Sinha, 1980; Jaiswal, 1986; Bilgrami and Sinha, 1987), pulses (Vora, 1978), oil seeds (Rao et al., 1965; Subramanyan and Rao, 1974; Basappa and Shreenivasamurthy, 1974; Dubey, 1980; Prasad and Sahay, 1987) and spices (Seenappa, 1970; Samajpati, 1983; Prasad et al., 1986). Bhat et al. (1978) resumed the work done in several states in South India and reported many of the places as high risk region for mycotoxin development on products of food importance. Literature indicates that only a few reports have been made from Central India on this important aspect, though the climatic conditions in this region are quite suitable for mycotoxin contamination in various crop produce (Jain et al., 1992). Literature also indicate that only
a few reports have been made on the cytotoxic effects of mycotoxins in plant system (Bilgrami et al., 1986). Whether, the substrate like seeds of various crops which carry mycotoxin(s) are also get damaged by their presence, is a subject of investigation, because of the well established carcinogenic and mutagenic properties shown by some mycotoxins on animal system.

Taking the broadest possible view of the subject, present study was planned to investigate the extent of mycotoxin contamination in some common food grains and animal feed in the Sagar district of Madhya Pradesh. Association of fungal contaminants with such product is also equally important and has been worked out by carrying a survey in this region. The effect of fungal metabolites and aflatoxin $B_1$ on seed germination, seedling vigour and on cell division process have also been evaluated and discussed.