CHAPTER - 1

INTRODUCTION
INTRODUCTION

Tea of commerce [Camellia sinensis (L.) O. Kuntze] is extensively cultivated in the North East India since the beginning of the 19th century and it has become the most popular beverage world over. India is the leading country for the production, consumption and export of quality tea all over the world. India has 4,32,297 hac area under tea cultivation with a production of 8,10,613 billion Kg. In 1997 of which North East India contributed 4,998.9 billion kg. Cachar’s contribution has been 48 million Kg during 1997 from 35,000 hac. area under tea. It has been reported that the common mould observed on made tea in the tea factory premises were Aspergillus flavus Link and other Aspergilli and Penicillia (George et al., 1994). The possibility that tea may serve as a vehicle for the pathogens has been reported earlier (Ekanayaka et al., 1987).

The information available on the air spora of tea plantations of Assam is fragmentory. Also, very little information on the factory atmosphere is available so far. The only available data on tea air spora is from the Tocklai Experimental Station of TRA, in their annual reports published since 1967 onwards. It was further worked out by Devi (1984) that basidiospores and ascospores of Corticium spp. constitute a major component over the tea field. It was possible to forecast the disease development to issue suitable and fairly accurate warning, to be ready for spraying for controlling the disease effectively.
Similar work was reported by Dutta and Debnath (1990) on "Blister blight disease" of tea in relation to forecasting and management of the disease in Darjeeling, where it has been a serious problem.

Studies on aerobiology helps to study the air spora both in outdoor and indoor environment. These studies help to understand the source, take off, passive transport, deposition and impaction of these microorganisms. Air borne microorganisms are almost ubiquitous. The composition of this microflora depends on its source, whether from the structure of superficial colonozation of the building, from humidifiers, or from materials being handled, the conditions for microbial growth, the amounts of activity and ventilation. Many of the problems are not new but gaining importance because of the serious damaging nature of the toxins released by the microorganisms (i.e. mycotoxins) in consumable food items and beverages (Lacey, 1994).

Among the various moulds that grow on coffee seeds, certain species of the genus Aspergillus and Penicillium have been known to produce certain substances collectively called fungal toxins or mycotoxins. During the last few years, ochratoxin, one of the mycotoxins produced in coffee has received considerable attention. In commercial green and roasted coffee samples tested in Europe (which have originated from different countries of the world), varying levels of ochratoxins have been detected. (Ramesh and Vasanthi, 1998)

Ochratoxins have been known to occur in a variety of food such as barley, wheat, soyabean etc. Ochratoxins containing food when fed to laboratory animals cause toxicity symptoms and damage of the kidneys. In some countries
in Europe like Denmark, former Yugoslavia and Bulgaria, pigs suffer from a
disease called porcine nephropathy. Ochratoxins were detected in the biological
samples such as blood, urine, liver and kidney of these animals. In these countries,
humans also suffer from a disease called Balkan Endemic nephropathy.

Aflatoxins are carcinogenic substances produced by the fungi,
*Aspergillus flavus* and *A. parasiticus*, which have received worldwide attention
for the last three decades. Their affect on human and animal health have been
well documented. Occurrence of aflatoxins in food and feed has been a problem
in many countries. Corn is one of the most susceptible crop to aflatoxin
contamination, and approximately 70% of corn stocks in both private and
Government ware-houses were estimated to be contaminated with non-tolerable
levels (over 20 µg/kg) of the toxin (Bilgrami et al., 1980).

Post harvest handling and treatments for agricultural products have
become the most important issue in the countries where the mycotoxigenic fungi
are distributed widely. Aflatoxin examination for imported commodities takes
priority in the countries of temperate regions where aflatoxin producers are
uncommon in the farm fields. The control of mycotoxins produced by plant
pathogens might be different from those by storage fungi (Aibara, 1990).

The mycotoxins are produced as secondary metabolites by a wide
variety of fungi growing on grains, oil seeds, nuts, spoiled fruits, standing forages
and stored foods and feeds (Pier, 1991; Wyllie and Morehouse, 1977). Their
natural occurrence, seasonal variation, regional prevalence and substrate
preference create several scientifically important problems in human and animal
health. The effects exerted by mycotoxins on mechanisms of immunity and resistance have been the subject of several reviews (Pier et al., 1979, 1980; Richard et al., 1975, 1978).

Three groups of mycotoxins are prominently associated with immunosuppressive activity (Pier and McLoughlin, 1985), namely the aflatoxins, ochratoxin A and certain of the trichothecene toxins. Much of their toxicity is referable to their inhibition of protein synthesis, but each acts at a different site of protein formation. Aflatoxin binds to DNA, suppresses DNA-dependent RNA production and thus interferes with transcription (Clifford and Rees, 1966; Hsieh et al., 1977). T-2 toxin, representative of several of the trichothecene toxins, affects initiation of translation in protein production (Hsieh et al., 1977; McLaughlin et al., 1977). Ochratoxin A inhibits protein synthesis (McLaughlin et al., 1977). Current information indicates that these mycotoxins affect immunogenesis in different ways.

From an economic standpoint of view approximately 25% of the World's crops are annually affected by mycotoxins (Cast, 1989). This equates to a direct cost of billions of dollars due to loss of crops and animals plus the hidden indirect costs incurred in monitoring the level of aflatoxins in crops and decreased performance of farm animals that ingest aflatoxin and other mycotoxins (Trail et al., 1995).

Aflatoxins have been the subject of numerous reviews in the past few years covering ecology (Cotty et al., 1997), occurrence (Jelinek et al., 1989), detection (Pestka, 1986, 1988), effects on human health (toxicity, carcinogenicity)
(Bray and Ryan, 1991; Chang et al., 1994; Chu, 1991; Dvorackova, 1990; Eaton and Gallagher, 1994), bio-synthesis (Bhatnagar et al., 1992, Dutton, 1988) and compounds which affect biosynthesis (Zaika and Buchanan, 1987), and control of aflatoxin contamination (Park et al., 1988; Park and Liang, 1993; Bhatnagar et al., 1995).

Aflatoxin B$_1$, B$_2$, and G$_1$ and G$_2$ are revealed by two 4 KV fluorescent spots. One compound fluoresced blue-violet hence called aflatoxin B and other having lower Rf value, fluoresced green was called aflatoxin G. Further B and G are differentiated into B1, B2 and G1 and G2 on the basis of their Rf values. The melting points of B1, B2 and G1 and G2 range from 240-269 °C and the thermal processing does not destroy aflatoxin (Manoharachary, 1986).

Aflatoxin is the potential toxin carcinogen, taratogen and mutagen. Aflatoxin B1 is known to have the greatest biological activity, while M1 has a significant biological potency. Due to intake of more potential aflatoxin by susceptible animals, impairment of liver function, necrosis of Kidney tubes, damage to lymphoid organs and other such effects on animals have been reported.

The statistical analysis of human epidemiological studies reveals that the intake of potential aflatoxin (AFB1) in higher dosage by susceptible human can result in liver cancer and liver cirrhosis. It is believed that aflatoxins being hepatocarcinogen induce similar tumors, with variations in the type of cells involved. Ingestion of aflatoxin relating to hepatoma were shown covering various regions in African and Asian countries (Monoharachary, 1986); Krishnamachari et al
(1975) have shown that out-break of hepatities in some Asian Countries is due to aflatoxin.

The Ochratoxin is a group of secondary metabolites produced by species of *Aspergillus* and *Penicillium*. The Ochratoxins have been designated as A, B, C and D belonging to methyl and ethyl esters (Monoharachary, 1986).

Aflatoxin B<sub>1</sub> has been regarded as the most toxic followed by G<sub>1</sub>, G<sub>2</sub> and G<sub>3</sub>. (Monoharachary, 1986). Liver is the major part that is affected but changes do occur in many other organs. Carcinogenic activity of aflatoxin has been demonstrated in ducks, trout, rats and ferrets. Enomoto and Saito (1972) have expressed that aflatoxin B<sub>1</sub> may be responsible for liver cancer in human beings and animals.

Prevention, removal and detoxification are the three basic principles involved in aflatoxin control. The production of aflatoxin can be avoided if effective storage conditions are practised. The growth, development and microbial infestation under storage conditions can be controlled by modifying the interseed environment and by the use of chemical preservatives. Prevention includes the usage of improved and mould resistant varieties. Aflatoxin contaminated commodities can be processed for detoxification by employing large number of physical, biological and chemical methods (Goldblatt, 1969).

Fungicides, insecticides and several chemicals have been screened for the prevention of mould growth in stored grains, foods and feeds. Chemicals used in foods or feeds as preservatives or stabilizers were screened
for prevention or reduction of *Aspergillus flavus*, *A. Parasiticus* and other toxigenic moulds. Drying the grain to less than 13.5 percent moisture content, low temperature, storage of grains, treatment of grains in storage with propionic acid and its sodium-calcium salts have been employed as preventive measures. Irradiation and UV Light treatment have been reported to degrade aflatoxins. Certain chemical compounds and plant extracts are known to inactivate aflatoxins by modifying their structure to non-toxic or less toxic compounds as detoxifying agent. These are methylanin, ethanolamin, choline and soda alone. Many microbes have been tested for their capacity to degrade, aflatoxins. (Monoharachary, 1986).

Understanding the interactions that occur between microorganisms is now insufficient, we must be able to modify these interactions too, If we do so then future awaits the exploitation of microbial antagonism to control the aflatoxin producing organisms.

There is evidence that aflatoxin can become air-borne and circumstantial evidence suggests that aflatoxin may also occur in the air. Their inhalation can be up to 40 times greater than by ingestion (Craesia et al., 1987). Ingested and other toxins may cause liver cancers; while other mycotoxins can affect other organs and interfere with the body defence mechanisms.

Mycotoxins are proved to be secondary metabolites produced by the activity of fungi on agricultural commodities; these are products that become poisonous and elicit a toxic response known as mycotoxicoses when food or feed containing them is eaten by human beings or animals (Monoharachary,
Mycotoxins are produced by a variety of moulds in staple food such as corn, wheat, rice etc. Aflatoxins are particularly known to be produced by the Aspergilli (i.e. patulin, ochratoxin etc.)

Many common features in epidemiology, kidney structure and kidney function in human suffering from Balkan endemic nephropathy and porcine nephropathy in pigs have been observed. In countries like former Yugoslavia, Poland, Germany, Denmark, Bulgaria and even Canada, ochratoxins have been detected in human blood samples. In Germany and Italy, ochratoxins have been detected in human milk samples.

The major source of exposure to ochratoxins by human in European countries is through the consumption of pork muscle, fat and kidney, poultry muscle and dairy products. Studies carried out in England have indicated that for humans, the contribution of ochratoxin intake from coffee is not more than 2% of the intake from all other foods. This clearly indicates that the danger of ochratoxin to human health from coffee is negligible.

In future, the presence of ochratoxins in coffee will be tested by the importer and it is found to be present above certain limits, the lots will be rejected leading to a heavy economic loss to the exporters (Ramesh and Vasanthi, 1998). Hence, it is essential to take effective measures at the estate, curing works and subsequent storage levels in order to minimise the problem of ochratoxins.

The mould damage occurs mostly because of the higher than 12% moisture level in coffee. Then chances of fungal contamination are more in coffee
berries dried in mud floor than in those dried on clean concrete/tiled floor. During
drying constant stirring and raking is essential. Rehydration of coffee should be
avoided. The practice of heaping cherries for 2-3 days should be discouraged
since the heat generated will encourage mould growth and subsequent toxin
production. Cuts, bits, bruised beans are more susceptible to mould damage
than whole beans and hence they should be sorted out. Heaping of parchments
immediately after washing for a couple of days could also result in mould growth
and hence should be avoided (Ramesh and Vasanthi, 1998).

There is no satisfactory measure to prevent the mould growth and
detoxification of the contaminated commodity. It is therefore, urgently needed to
investigate mycotoxin problems in edible commodities in diverse geographical
regions and to develop effective and economical method for controlling the mould
growth and subsequent production of mycotoxins.

The microbial antagonism that is seen in biological control of plant
pathogens is broadly based on the categories of competition (for nutrients and
space), parasitism (which may be the production of volatile or nonvolatile
antibiotics), and hyperparasitism. All of these mechanisms may operate together
or independently, and their activities can result in the suppression of interaction
in turn and to relate these mechanisms to examples of successful biological
control in field. Deb & Dutta (1991) reported the biological control of foot rot
disease of soyabean caused by Sclerotium rolfsii with the help of Trichoderma
spp. (i.e. T. viride and T. harzianum)
Competition for nutrients and space, the production of antibiotics and hyperparasitism play important roles in the antagonism of pathogens arriving and persisting in the phyllosphere (Blake man and Fokkema, 1982; Blakeman & Brodie, 1976; Kranz, 1980) Epiphytic microorganism take up nutrients rapidly, resulting in rapid reductions in the amounts of nutrients available to pathogens (Brodie & Blakeman, 1976; Fokkema, 1981) Under these circumstances, spores of Botrytis cinerea, Cladosporium herbarum, and Phoma betae do not germinate or they germinate poorly, failing to give rise to infections. Fungal Species are reported as important in antagonism on the phylloplane, particularly species of Trichoderma spp. (Singh, 1986; Sundheim, 1985).

Competition occurs when there is demand by two or more microorganisms for the same resource in excess of the immediate supply. The term can be used broadly to denote factors favouring one species over another or in a narower sense it can mean the active demand in excess of material or space on the part of two or more organisms (park, 1960, Waksman, 1952).

Clark (1965), defined competitions as the injurious effect of one organisms on another because of the utilization or removal of some resources of the environment. These resource can include nutrients, oxygen and space. Species that prefer the same ecological niche usually avoid competition and occupy different geographical areas or different habitats in the same area. Space and oxygen are probably important variables that can change in the rhizosphere or rhizoplane to the detriment of pathogen establishment, in that the occupation of a site by microorganisms must diminish the limited supply of nutrients in either
environment. According to Baker (1968), carbon, nitrogen, and vitamins are all important in this respect because they determine the growth and infection of soil-borne plant pathogens in competition with other organisms.

"Black rot" is a devastating disease of tea which is predominantly known to occur in the Cachar District of Assam. It is responsible for the heavy loss of this cash crop. A thorough and systematic study of the same is essential to carry out in this region (Cachar District). It is high time to follow the integrated pest management in order to control some important tea diseases. Hence an attempt has been made in the present study to understand the possibility of using some of the antagonistic fungal species from the tea atmosphere, phyllosphere and soil for the control of Black rot disease of tea caused by *corticium invisum*.

Integrated pest management (IPM) is being practised now-a-days in order to avoid the disastrous impacts of environmental pollution caused by the organic/inorganic chemical pesticides. It has been a subject of growing concern to both environmentalists and public health authorities. The study of antagonistic pathogens may lead to the proper understanding and application in the field condition, so that an ultimate goal of biological control can be achieved. It is high time to apply the knowledge of integrated pest management to a great extent in order to get rid of the pollutive effects of the pesticides.

Tea is one of the most important beverages produced by India and known to carry various mycoflora which may secrete toxins. No systematic study has been made on the same till date, especially under the agroclimatic
conditions of Barak valley. Therefore an attempt has been made to investigate the same in the present work. A proper investigation on the mycotoxins produced on the beverages (i.e. tea) and to achieve contamination free beverage (i.e. tea) is required to be worked out. These aspects have been given due attention in the present work.

The present study concerns with the air mycoflora of tea plantation and factory with special reference to the aflatoxin producing ability of the indoor mycoflora (Tea factory). The present work also includes the biological control efficiencies of the tea field, air and phyllosphere mycoflora.

In the light of the above facts the present investigation was, therefore, undertaken with the following objectives:-

1. A comparative study of the mycoflora in air, soil and phyllosphere.

2. Observation on the seasonal variation of the tea factory air mycoflora.

3. Study of the aflatoxin producing potential of some of the isolated mycoflora from the tea factory atmosphere (i.e. from fermentation room, drying room and sorting room), phyllosphere and soil.

4. Study of the biological control potential of some of the tea phyllosphere mycoflora against the black rot disease causing organism i.e Corticium invisum.

5. SEM study of some of the mycotoxin producing mycoflora and biocontrol agents (antagonists)

6. Statistical analysis of the data obtained.