Introduction

Tea with its own characteristc flavour as well as stimulating and soothing qualities, is drunk all over the world. The use of an infusion of dried tea leaves has its origin in antiquity. Available statistics show that the total amount of world tea production reaches 2.5 million tons annually and an average of 2 billion cups of tea are drunk everyday (Yamanishi, 1991). In India, tea is grown in two distinct regions - the North Indian tea belt located between 22-27° N, characterzied by wet and hot summer and a cold, dry winter, and the South Indian tea belt located at 7°N latitude which enjoys a tropical climate (Jain, 1991). Tea cultivation forms the backbone of the economy of North East India and it produces 75% of the total Indian tea in three different landscapes. The extensive alluvial reverine flat plains at the base of Himalayan ranges are the tea districts of Dooars and Terai. Darjeeling produces the world's finest quality teas in the steep slopes of Eastern Himalayas upto an elevation of 2000 m. Brahmaputra valley of Assam is located 100 m above sea level is the largest flood plain of the world growing tea and this accounts for more than half of Indian production. South Indian tea is grown in the slopes of Nilgiris and Annamalai hills.

The tea plant [*Camellia sinensis* (L) O. Kuntze] is a perennial and being cultivated in such widely differing areas, a large number of varieties have evolved, each adaptive to grow in the specific region. Alongwith this adaptation, changes have also occurred in their flavour characteristics, growth patterns and response to pathogen attack. Plants, in general, are constantly exposed to millions of microorganisms and many of these microbes possess the faculty to attack the plants. Being a perennial ,the tea plant possibly interacts with, and samples more environmental problems than do most other plants. Any damage to the leaves of tea plant causes enormous economic loss as it is the leaves themselves which are used for beverage production. Foliar diseases of tea thus assume great economic significance. Grey blight, caused by *Pestalotiopsis theae* (Sawada) Stey. is one of the foliar diseases of tea prevalant throughout North East India (Agnihothrudu, 1995). Symptoms of the disease generally appear as light to dark brown patches with a greyish centre on the upper surface, roughly circular to oval, marked with concentric zonations almost from the centre to the very edge. Black pustules of the sporodochia are produced in concentric lines on the upper surfacer (Plate I, figs. A-C). On young leaves, the patches are usually dark brown to almost black, rather irregular in shape and not marked with concentric rings. On older leaves the patches lead to the breaking away of the lamina into irregular species (Plate I, fig.D) and in severe cases, the fungus can considerably reduce the maintenance foliage leading to debility of the bush and die back. In Japan *Pestalotia longiseta* Speg. is reported to cause grey blight which is considered to be an important problem (Yanase and Takeda, 1987). Another species of *Pestalotiopsis, P. versicolor*

which is considered to be an important problem (Yanase and Takeda, 1987). Another species of *Pestalotiopsis, P. versicolor* has been found to exist in South India.

In nature, unlike animals, plants cannot move and hence, they cannot side step or run away from the attacking pathogen. They are bound to stand, wait and then face the attacker. In order to suit the above situation, plants have evolved a primarily localized type of defence or immune system, where each individual cell /tissue/organ is required to defend itself without much help from distant plant parts (Singh and Singh, 1988). A pathogen is recognised and restricted by the individual or few plant cells. An organism is recognised at the level of surface to surface interaction as either incompatible or compatible. Recognition of interaction as the compatable one depends on some kind of molecular similarities between the host and pathogen (Heide and Swedegaard - Peterson, 1985 ; Chakraborty, 1988 ; Protsenko and Ladyzhenskaya, 1989 ; Chakraborty and Saha, 1994b). Close serological similarity betwen host and pathogen is therefore one of the prerequisites for the successful establishment of the pathogen in its host.

Taking advantage of the serological relationship between host and pathogen, the antiserum raised against the pathogen is being used for the detection of the pathogen in the host tissues beginning from the early stages of host pathogen interaction (Mohan, 1988 ; Linfield, 1993) as well as from soil (Wakeham and White, 1996). Commercial diagnostic kits have been offered in recent years for the rapid diagnosis of several fungai in plant tissues, soil and water (Werres and Steffens, 1994). Targets for kits include *Phytopthora sp., Pseudocercosporella sp. , Pythium sp. , Rhizoctonia sp. , Sclerotinia sp.* and *Verticillium dahliae.* However, little is known about factors influencing the test reaction of kits. Some kits cannot distinguish among individual fungal species and show some cross reactivity (Ali-Shatayeh *et al.,* 1991). To obtain a reliable diagnosis, suitable thresholds considering all possible influencing factors need to be determined (Miller *et al.,* 1988; MacDonald *et al.,*1990).

Considering the importance of grey blight disease of tea, and the fact that studies on the serological relationship of *Pestalotiopsis theae* and tea are lacking , the study has been undertaken with the following objectives : (a) screening of tea varieties obtained from Tocklai, Darjeeling and UPASI Tea Research Centres for resistance to *P.theae* ; (b) extraction of antigen from mycelial and cell wall preparations of *P.theae* ; healthy, artificially inoculated and naturally infected tea leaves; non host species and non pathogen ; (c) raising of antisera against antigen preparations from tea leaves, mycelium and cell wall of *P.theae* as well as of *F.oxysporum* ; (d) detection of serological cross reactivity between *P.theae* and tea

varieties following agar gel double diffusion, immunoelectrophoresis and enzyme linked immunosorbent assay; (e) optimization of the sensitivity of ELISA using antisera raised against both cell wall and mycelial preparations of *P.theae* ; (f) detection of infection in tea leaves of different varieties by ELISA ; (g) determination of the earliest period after inoculation with *P.theae* , when detection is possible by ELISA and quantification of the fungal biomass in tissues; (h) purification of the antigenic protein (s) from the crude mycelial extracts of *P.theae*; (i) evaluation of antiserum raised against partially purified antigens of *P.theae* ; (j) characterization of chemical nature of cell wall of *P.theae* ; (k) determination of the cellular location of cross reactive antigens in tea cells and leaf tissues, mycelia and conidia of *P.theae.*

Before going into details of the present work, a brief review in confirmity with this study has been presented in the following pages.

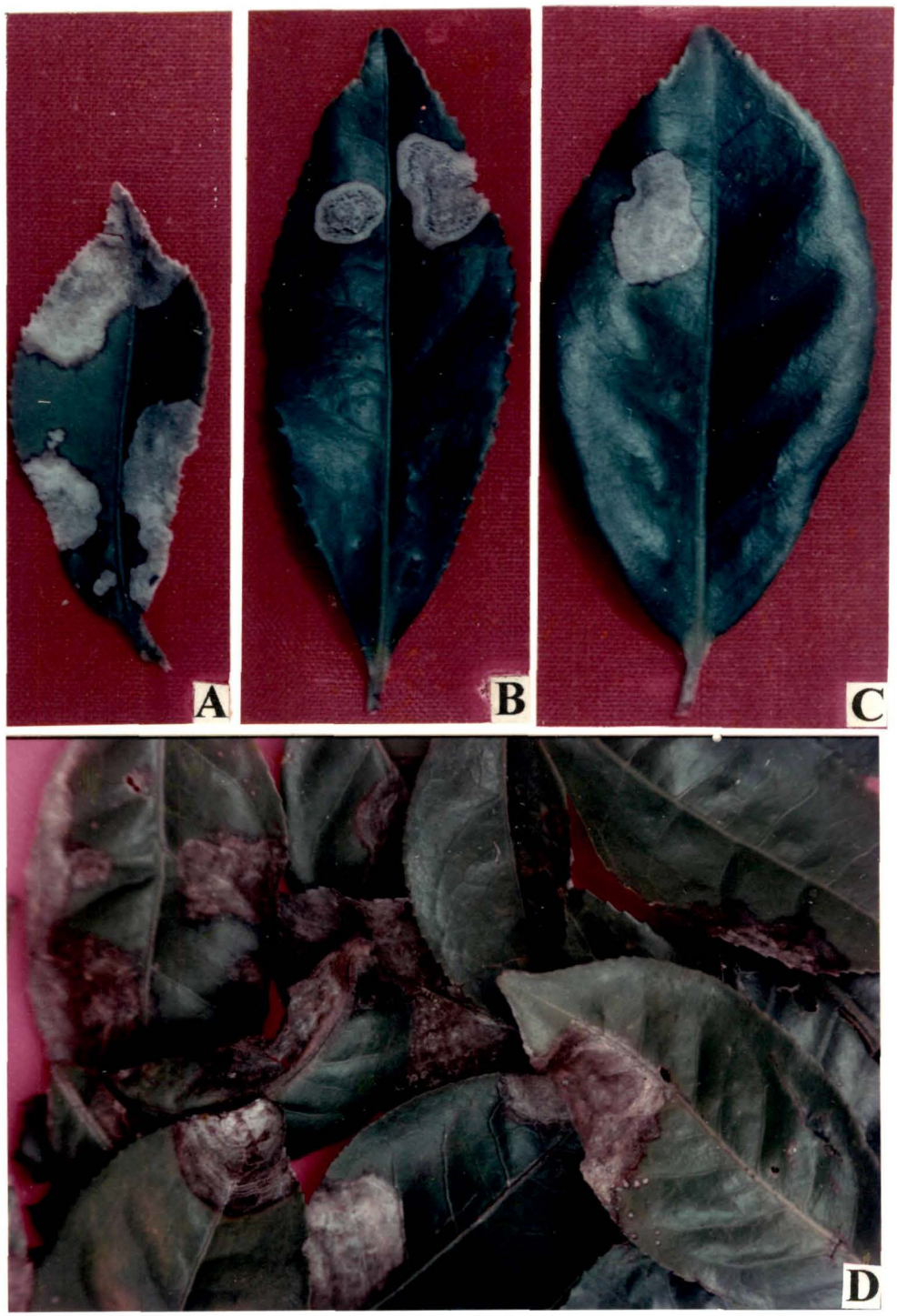

Plate I (figs.A-D) . Tea leaves naturally infected with the grey blight pathogen, *Pestalotiopsis theae*