INTRODUCTION

Tea [*Camellia sinensis* (L) O. Kuntze], which originated in China, boasts the world's longest history among beverages. Offering its own individual flavour as well as soothing and refreshing qualities, it is drunk all over the world. The tea industry has spread from China to the far corners of the world, currently encompassing countries ranging from 30° latitude in the southern hemisphere to 45° latitude in the northern hemisphere, and from 150° longitude in the east to 60° longitude in the west. In India, tea is one of the most important cultivated crops and is grown in two distinct regions- the North Indian tea belt located between 22-27° North and South Indian tea belt located at 7° North. North East India produces 75% of the total Indian tea in 3 different land scapes (Jain, 1991). Bramhaputra valley of Assam located 100m,above sea level is the largest flood plains of the world growing tea which accounts for more than half of Indian production. Darjeeling produces the world's finest quality teas in the steep slopes of Eastern Himalayas up to an elevation of 2000m.The extensive riverine flat plains at the base of Himalayas are the tea districts of the Terai and Dooars. Tea is also grown in the slopes of Nilgiris and Annamalai hills of peninsular South India.

The tea bush, like any other living plants is susceptible to attacks by pathogen, more so as it has been forced to grow, under varying climatic and soil conditions remote from its natural environment. For the benefit of mankind it has also been subjected to varied cultural treatment which are widely at variance with its natural conditions of growth. Diseases of the tea bushes and its ancillary crops may be caused by one or more of several agencies viz.,(a)vegetable organisms, such as fungi, algae and bacteria, (b) animal parasites, (c) adverse conditions of soil and climate, (d)mechanical damage and (e)virus.

Brown root rot disease of tea caused by *Fomes lamaoensis* (Murr.) Sacc. & Trott. is one of the primary root diseases found on all soils but more common on sandy soils. The pathogen attacks the tea plants from about 3years upward but younger plants may easily be attacked and killed if their roots happen to come in contact with diseased material in the soil. The above ground symptoms appear gradually, which includes

withering and bending of leaves and young branch-tips and losing of moisture content. Diseased plants die suddenly at advanced stages and their dried leaves remain attached for some time. Green colour of leaves changes gradually towards pale or ash green.

Roots of affected bushes are encrusted with soil, sand and stone particles held firmly by a brown mycelium which is difficult to remove by a good washing or rubbing and it sometimes forms blackish sheet on root surface. Brown or soft mycelium grows on collar region, extending on the main stem to a few inches. A thin film of white or brownish mycelium may develop on the surface of affected wood underneath the bark. In advance stages irregular rings and reticulations are formed by hard brown or blackish lines on/in the affected wood. Colour of wood is light yellow or brown and wood becomes soft. Sometimes the soft wood inside the brown rings decays and slices out, giving a honey-comb-like structure (Plate 1).

Plants respond to infection by pathogens in a number of ways, which are triggered by the initial recognition phenomenon. The initial recognition triggers the activation of immune systems of plants, which though different from that of animals, is functionally similar. Immune system in plants involves several mechanisms, some of which are known to us, but many of them are still unknown. An important area of immunological studies of disease involves the use of pathogen antiserum for detection of infection in the host beginning from the very early stages of host pathogen interaction. Disease detection by immunological means is gaining ground incase of fungal diseases (Chakraborty and Chakraborty, 2002). The recent diagnostic techniques for pathogen detection include enzyme linked immunosorbent assay, dot immunobinding assay, western blot analysis, immunofluorescence and immunoenzymatic staining. Though significant advances have been made in the development of rapid, sensitive assays for fungi in recent years, commercially available techniques are limited to a few pathogens and diseases. Such detection techniques makes it possible to detect microquantities of the pathogen within a few hours of infection, which is much more advantageous than the conventional techniques involving pathogen inoculation, visible symptoms and microscopy. These have tremendous potential for plant disease control measures since detection of a pathogen at the initial stages of infection can lead to

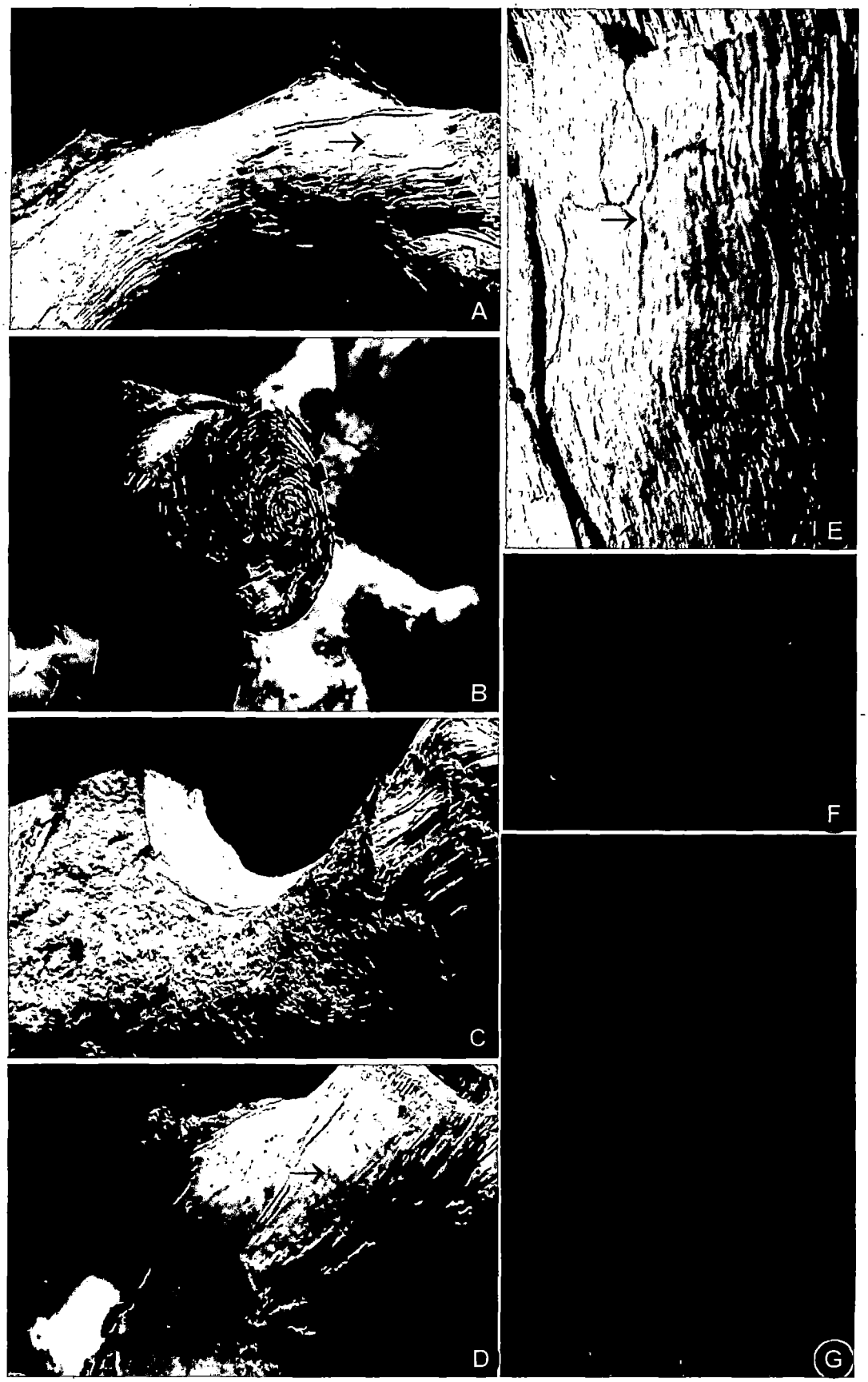

Plate 1 (A-G): Naturally infected tea root showing symptoms of brown root rot disease

formulation of control measures before much harm has been done. 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; Chakraborty *et.al.* 1995), as well as from soil (Wakeham and White, 1996).

Indian agriculture is now revolving under bio-prospective bioscience orbit. Biofertilizers, biopesticides, biological control and bioremediation are considered the four pillars of bioprospective bioscience. Adopting biological practices for a preventive approach of control diseases is at present on focus. So, for control of root diseases of tea, the most environmental friendly and effective one is the introduction of biocontrol agents in the soil among the current management practices and thus minimizing the use of fungicides.

Trichoderma spp. is a potential biocontrol agent for control of soil-borne diseases and is the common antagonist found in all the soils. The biological control of important root diseases of crop plants like tea and others like rice, millet, sunflower and maize can effectively be achieved using *Trichoderma* spp (Chakraborty et al.2002a). The efficacy of *Trichoderma* spp. as biocontrol agents of groundnut stem rot and root rot diseases was reported (Sreenivasaprasad and Manibhushanrao, 1993; Biswas and Sen,2000). Sunflower seed pathogen could also be controlled by *T. harzianum* (Janardhana, 1994).

For effective biological control, efficient and well-designed formulations are essential to reach the target of successful disease control. Agricultural wastes such as saw dust, tea wastes and wheat bran proved to be the best medium for sporulation and mass application. Sivan et al., (1984) found that a mixture of peat and wheat bran was a much better substrate.In another study, application of wheat bran saw dust (WBSD) preparation of *Trichoderma* spp. has been useful to control several soil-borne, root-infecting pathogens under field conditions (Baby and Manibhushanarao, 1993).

Considering the importance of tea as a plantation crop in this area, along with

all the above, the present study has been undertaken with the following objectives:

(a) Varietal resistance tests of tea clones against *Fomes lamaoensis* causing brown root-rot disease.

(b) Preparation of mycelium and cell wall antigens of *Fomes lamaoensis* and production of polyclonal antibody.

(c) Purification of antigens and antisera and analysis by immunoblotting.

(d) Detection of serological cross reactivity among tea roots and fungal isolates.

(e) Detection of *F. lamaoensis* in soil and tea root tissues using ELISA, immunofluorescence tests and immunocytochemical staining.

(f) Quantification of fungal biomass in tea root tissues and soil using various format of ELISA, Dot Blot and Western Blot.

(g) *In vitro* interaction studies with *Trichoderma harziamun*, *Trichoderma viride* and *F. lamaoensis.*

(h) *In vivo* test with *T. harzianum* and *T. viride* for management of brown root-rot disease.

The materials used and methods applied to achieve the above objectives have been outlined in the following pages along with the results achieved. In the beginning a review of the literature along lines pertaining to the present work has also been presented.