INTRODUCTION

Tea [*Camellia sinensis* (L) O.Kuntze], is one of the most important plants from the economic viewpoint and being a perennial is always challenged by pests and pathogens, which provides a stable microclimatic conditions as well as supply of food for rapid build up of insects. Tea boasts the world's longest history among beverages and offers more beneficial than being a soothing beverage to be served at social gathering. Offering its own individual flavor as well as soothing and refreshing qualities, it is drunk all over the world. There is mountain evidence to suggest that drinking tea may also reduce the risk of developing cardiovascular disease and many forms of cancer. 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. Darjeeling produces the world's finest quality teas in the steep slopes of Eastern Himalayas up to an elevation of 200m. The extensive reverine flat plains at the base of Himalayas are the tea districts of the Terai and Dooars.

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 like (i) vegetable organisms, such fungi, algae and bacteria, (ii) animal parasites, (iii) adverse conditions of soil and climate (iv) mechanical damage and (e) virus. Charcoal stump rot disease of tea caused by *Ustulina zonata* (Lev.) Sacc.. is one of the primary root diseases occurs on all soils. It is found in all the tea growing areas and is probably the commonest of all the primary root diseases of tea in North-east India. It attacks tea bushes preferably more than three years old. The plants being perennials harbour the pathogen in root tissues and rhizosphere, over a long time. Diseased bushes, especially the young ones, die suddenly but their withered leaves remain attached to the main plant for sometime. Sometimes older bushes may be dead and rotten one side while other side remains apparently healthy for a long time. The disease results in crop losses. The fungus develops a characteristic fructification, at first white changing to charcoal-like black,

Plate 1: Tea plantations in the North Bengal University Campus.

brittle encrustation, which is wavy on the surface. Charcoal like, brittle wavy encrustations on the bark and exposed wood at the collar region appear as external symptoms.

The root surface usually bears small, white or black, isolated, cushions or lumps. Fan-like felts of dull white, silky mycelium grow on the surface of diseased wood, underneath the bark. Colour of the wood become dull-white, almost normal and permeated by irregular, single or double, black bands or lines (Plate 2). The disease spreads mainly by direct contact between infected and non-infected roots. Bits of left over infected root pieces after uprooting serve as potential sources of infection .

There is cell surface interaction between the host and pathogen, either as compatible or incompatible. Recognition or interaction as compatible depends on some kinds of molecular similarities, between the host and pathogen (Chakraborty, 1998). Close serological similarity between host and pathogen has been found to be one of the prerequisites for the successful establishment of the pathogen in the host. This serological assays have also been exploited for the development of immunodiagnostic kits for pathogen detection systems in the host. Such disease detection and diagnostic kits have the advantage over conventional methods by being specific and having the ability to detect even minute amounts in tissues. Commercial diagnostic kits have been offered for the rapid diagnosis of several fungi in plant tissues, soil and water (Werres and Steffens, 1994, Chakraborty and Chakraborty, 2003, Gawande *et.al*, 2006). Most common among these techniques are ELISA, Dot-blot, Western blot, indirect immunofluorescence, immunocytochemical staining used in large scale disease indexing programmes in perennial and vegetatively propagated crops. Timely detection of disease especially root diseases combined with proper management practices can lessen crop loss to a great extent.

The defense strategies of plants against their pathogens are manifold and include the use of antifungal chemicals. On the other hand, pathogens have evolved mechanisms to evade these chemicals. In such relationship it has long been recognized that responses are characterized by the early accumulation of phenolic compounds at the infection site and that limited development of the pathogen occurs as a result of rapid cell death. Numerous studies suggest that low molecular weight phenols, such as benzoic acid and phenylpropanoids are formed in the initial response to infection. Most research on resistance mechanisms has shown that the plant uses defenses that are activated after infection to stop pathogen development. Tea plants

Plate 2: Naturally infected tea root showing symptoms of charcoal stump rot disease.

are abundant source of flavonoids, a group of compounds with antioxidant properties of which specific interest are the flavonoids catechins and flavonols which prevent the synthesis of peroxidase and free radicals, agents that can invade cell membrane and damage genetic material. Since polyphenol are major constituents of tea leaves, their involvement in the defense mechanism either as preformed or induced chemicals seemed highly probable.

Biocontrol agents are the most environmental friendly and effective. Among the available biocontrol agents *Trichoderma harzianum and Trichoderma viride* have been tested in a large number of cases. Biomass production, their suitable formulation for commercialization of antagonists to check chemical fungicides usage are being developed.

Biocontrol agents in soil amendments using oil cakes and dung manure found effective in controlling root diseases in French bean root rot and Lentil wilt complex .In that context, the possibility of disease control through integrated management (Biocontrol agents and organic residue materials), assumes much greater significance. Considering all the above the present study has been undertaken with the following objectives antibiosis.

The basic objectives of the present investigation are (a) screening of tea varieties for resistance to *Ustulina zonata;* (b) estimation of host parasite proteins before and after infection; (c) determination of the level of phenolics in tea roots following inoculation with *U. zonata;* (d) ascertaining the antifungal activity of phenolics associated with differential host response to infection; (e) assay of phenylalanine ammonia lyase, peroxidase and polyphenol oxidase activities in tea roots following inoculation with *U. zonata*; (f) raising polyclonal antibody against mycelia and cell wall antigens of *U. zonata* and tea roots; (g) detection of serological

cross reactivity between *U. zonata* and tea varieties using immunoassays (h) detection of *U. zonata* in artificially inoculated tea roots by PTA-ELISA,DAS-ELISA, Competetion ELISA, dot immunobinding assay, indirect immunofluorescence and immunocytochemical staining; (i) *in vitro* studies of *U. zonata* with biocontrol agents; (j) developing effective integrated management strategies against charcoal stump rot disease of tea.

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