# Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is one of the most important evergreen perennial plantation crops of India and most popular hot drinks in the world over. Since very ancient time habitual tea drinking has been considered to be beneficial to health. Tea is known to have antimutagenic, anticarcinogenic, antibacterial, anticoagulant, and having potent antioxidative properties. Its cultivation, spread over more than 3,96,000 hectors of land divided into two distinct regions –the North Indian tea belt region located between 22°- 27° N and South Indian tea belt at 7° N. North- East India produces 75 % of the total Indian tea in three different land scapes –the hilly terrain of Darjeeling upto an elevation of 2000 m, yielding the world's finest quality of tea,the extensive riverine flat plains at the base of Himalayan range i.e. Terai Dooars, (Plate I) and the Brahmaputra valley of Assam located at 100m. above sea level, which is the largest flat plains of the world and which accounts for more than half of the Indian tea production (Jain, 1991).Since the plant grown in the tropical agroclimatic zone, pest, weed and disease causing organisms cause serious damage to the crops, for which excessive use of chemical has been continuing since long past. Besides, as most of the nutrients in the soil remain in unavailable form so use of chemical fertilizer has been necessary for the optimum productivity of the crop.But the use of chemical fertilizers and pesticides have caused a serious problem of pollution and the loss of land fertility (Bezbaruah *et al.* 1996). Abiotic stresses are known to cause oxidative stress in plants by producing reactive oxygen species (ROS) like superoxide radical , hydroxyl radical, hydroperoxyl radical, hydrogen peroxide, which are inevitable products of natural redox reactions in various cellular compartments (Zhang and Kirkham 1994; Alscher *et al.* 1997 ).

Soil contamination with heavy metals released from anthropogenic activities has become a world wide problem, leading to the loss of crop yield and health hazards as they enter into the food chain (Salt *et al.* 1995; Schlickler and Caspi, 1999). Indiscriminate use of chemicals and the presence of residue in the tea leaves are a major concern and more sensitive issue than other crops to avoid toxic hazards for the consumers as they are harvested at short intervals. So, it is necessary to keep the

**Plate I:** Tea Garden in plains of Dooars

residues much below the Maximum Residue Limit (MRL) stipulated by different international agencies. Heavy metals are defined as the metals with a density higher than 5 gm $cm^{-3}$. Fifty-three of the ninety naturally occurring elements are heavy metals (Weast, 1984).Heavy metals are dangerous because they tend to bioaccumulate. The main sources of contamination in agricultural soil are fertiliser impurity ($Cd^{2+}$) , use of refuge derived compost and sewage sludge ($Cd^{2+}$,$Ni^{2+}$,$Pb^{2+}$,etc.) and to a lesser extent , mineral weathering (Hildebrand, 1989; Alloway , 1995). Cadmium can be found in soil because of insecticide, fungicide, sludge, and commercial fertilizers that are used in the agriculture field.Cadmium, the most toxic heavy metal pollutants for human beings, animals and plants as it enters in the environment mainly from industrial processes and phosphorous fertilizers and then get transferred to the food chain (Wagner, 1993). Agricultural plants represent an important pathway for the movement of potentially toxic trace elements from soil to human being. WHO (1984) has recognized health hazards of metal in food chain even at low concentrations (Nigam *et al.* 2002). When accumulated in the plant tissues, it causes alterations in catalytic efficacy of enzymes (Van Asseche and Clijsters, 1988; Somashekaraiah *et al.* 1992; Romero- Puertas *et al.* 1999.Piqueras *et al.* 1999), damage to the cellular membranes (Fu and Brouillette, 1997) and inhibits the root growth (Wilkins, 1978). These changes result in inhibition of chlorophyll biosynthesis and photosynthesis (Singh and Singh, 1997) and mineral nutrient uptake (Greger and Lindberg, 1987). Among various toxic metals, cadmium is recognized as the most hazardous element that is not essential for plant growth but easily taken up by plants (Nigam *et al.*2002).

Some of the heavy metals are essential for the life processes as trace elements, (copper, selenium zinc) to maintain the metabolism of the organism. Copper is a plant micronutrient that is an essential component of several enzymes and coenzymes involved in metabolic pathways of plants (Mourato *et al.* 2009). However, at high concentrations Cu can become phytotoxic affecting plant development due to direct or indirect interference with numerous physiological processes (Maksymiec 1997, Vangronsveld and Clijsters, 1994). At higher concentrations they interfere in plant growth and metabolism, induce perturbations in the root cell plasma membranes producing altered membrane structure and function, which result in nutrient deficiencies

in the leaf tissues (Palma *et al.* 1987, Demidchik *et al.*1997). Sources of copper toxicity in agricultural field are the use of copper containing fertilizers, fungicides and insecticides.

Stress is the physiological state of a plant when they are exposed to the extremely unfavourable conditions. The situation is counteracted by the alteration of the biochemical parameters. The extremely unfavourable conditions need not be fatal to the organism but will induce an "alarm response". It has been reported that oxidative stress induced by heavy metal toxicity leads to the production of reactive oxygen species (Aravind and Prasad, 2005). The reactive oxygen species (ROS) cause a variety of harmful effects in plant cells , such as inhibition of germination and photosynthetic activity , lipid peroxidation and DNA damage (MacFarlane ,2003; Maclecka *et al.*2001; Shaw *et al.* 2004). The ROS is scavenged in plants by antioxidant enzymes like super oxide dismuttase, peroxidase, catalase, ascorbate peroxidase, glutathione reductase and non enzymatic (carotenoid, ascorbate, glutathione) (Scandalios,1993). Measurement of activities of antioxidant enzymes is helpful in indicating the oxidative stress in plants (Geebelen *et al.* 2002). Cell death occurs when the oxidative stress induced metabolic change exceed the scavenging capacity.

As the tea plants are grown under monoculture, provide favourable conditions for variety of pests and diseases which accounts for the potential croop loss (Plate II). The use of pesticide in the field to combat pests and diseases has been advocated, which contribute to major anthropogenic addition to the natural communities. Indiscriminate and excessive uses of these chemicals create environmental pollution, bioamplification of the residue in human system and resurgence of pests and destroy the ecological balance by damaging target and non target organisms. The application of pesticide alters the physiological responses in tea by altering biochemical processes which would give them greater adaptability.The chemical fungicide and insecticide application also casuses the great phytotoxic effect to the plant. It also alters the plant metabolism and causes the oxidative damage to the plant. Some fungicides used to mitigate or prevent pathogen attack may be involved in activating certain defensive responses of plants by influencing the key steps of the phenolic and oxidative processes (Gracia *et al.* 2003). The chemical application like endosulfan, methomyl, acphate and surfactant at seedling

**Plate II:** Insect infested tea bushes.

stage alter the photosynthetic rate (Haile *et al.* 2000). The progressive loss of enzymic and non-enzymic antioxidants in plants subjected to pesticide treatment indicated a loss of cellular protective machinery with an increase in oxidative damage by increase in lipid peroxidation (Panda and Patra, 2000). The pesticide treatment can impose an osmotic stress causing damage to membrane structure. Membrane proteins interact with pesticides which may affect the production of enzyme protein (Deshpande and Swami, 1990) .Organophosphorous pesticides used in agriculture, by virtue of their anti-acetylcholinesterase (AchE) activity block nerve transmission and thus pose threat to non-target organisms including human populations (Somara *et al.* 2002).

Considering the importance of the above, the present work was undertaken to determine how the different tea varieties respond to the different chemical induced stresses.The objectives of the present work were –

1. To study the biochemical responses of different varieties to the stress in terms of changes in proteins, carbohydrates, proline, phenols, chlorophyll carotenoids.
2. To determine the effect of stresses on antioxidative enzymatic activities like peroxidase, phenyl ammonia lyase, polyphenol oxidase.
3. To determine the specific expression of new protein(s) following stress.
4. To determine the flavour component changes due to application of insecticide, fungicide or heavy metal.

In order to achieve the above mentioned objectives standard methods for the analysis have been used which have been described in the following pages. A brief literature review in this area has also been provided.