

# INTRODUCTION

Tea drinking is an ancient custom with origins in 2737 BC China. Today approximately 3,000 varieties of tea [*Camellia sinensis* (L.) O. Kuntze] are made from this single plant. Tea offers more beneficial than being a soothing beverage to be served at social gathering, or to ward off the chill of a winter's night. There is mountain evidence to suggest that drinking tea may also reduce the risk of developing cardiovascular disease and many forms of cancer (Chakraborty and Chakraborty 1998). Tea plants 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. Certain chemicals found in the molecular structure of these beneficial flavonoids, collectively known as phenolic groups. Since polyphenol are major constituents of tea leaves, their involvement in the defense mechanism either as preformed or induced chemicals seemed highly probable.

Tea is also 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 (Muraleedharan and Chen 1997). Among the insects, tea mosquito bug *Helopeltis theivora* Wat. (Hemiptera : Miridae) has assumed the status of major pests in several tea growing areas in West Bengal and Assam (Somchowdhury *et. al.*, 1993). Both adult and nymph of *H. theivora* suck the sap from the young leaves, buds and tender stem. Feeding by *H. theivora* is seemed primarily on the leaves. A circle is formed around the feeding spot within 2-3 hour of damage, in 24 hour the inside portion of the ring becomes translucent light brown in color and within a few days the spot turns dark brown. The tissue around the affected spots first turn brown and then black and subsequently they dry up (Plate 1). The dry leaf tissue in the long run dropped resulting in numerous perforations (shoot holes) on the leaf surface. A single leaf may have innumerable puncture marks. Severely damaged leaves also curl up apart from



PLATE 1 : Tea leaves naturally infested by *Helopeltis theivora* (inset)

ceasing to grow. The typical feeding damage by *H. theivora* appears as a discolored necrotic area around the point of entry. They feed at early morning, late evening and night hours.

The major fungal diseases of tea have been classified under the leaf, root and stem diseases. Among the foliar diseases of tea blister blight, black rot, brown blight and grey blight are important fungal diseases caused by *Exobasidium vexans*, *Corticium invisum*, *Glomerella cingulata* and *Pestalotiopsis theae* respectively (Agnihotrudu, 1995). The pests and blights which prey on the leaves are of vital importance, since any damage to the leaves defeats the whole purpose of its cultivation. Blister blight disease is very common in humid and foggy regions whereas black rot is common in doors and terai of North Bengal capable of causing significant depreciation in tea yield and quality. Serious infection causes defoliation starting from the center of the bush. Black rot affected leaves are characteristically greyish brown, but at a later stage of infection they become black particularly during the rains. The disease persists in the same areas for years, if not controlled, causing gradual deterioration in the health of the tea and loss of crop. Colour of the upper surface of the affected area at the early stage become reddish-brown, similar to sun-scorch damage, later it turns to a mixture of brown, yellowish-brown and grey the undersurface become light brown or grayish white and usually covered with a net work or cream to brown mycelium. The fungus produces on the stem, thick cords of mycelium, up to about 3 mm across, dark purplish-brown on the older portions of the stem and dull white to light brown on the green portions at the top. The fungus spreads not only by direct contact from bush to bush but also by wind, bird as well as by workers of the tea garden. Alternaria blight, another foliar disease of tea (Plate 2) caused by *Alternaria alternata* (Fr.) Keissler is very common in the nursery grown plants. Disease symptoms appear as greyish brown patches on the young leaves. Older leaves were less susceptible. Symptoms first appear in the tip region and the margin of the leaves, which extend towards the midrib following which the leaves curl, and die. It causes



Plate 2 : - Tea plant (Teen Ali 17/1/54) showing symptoms of leaf blight disease caused by *Alternaria alternata* (inset)

serious infection leading defoliation of leaves. It causes considerable damage to the plants maintained in the nursery as well as in the field (Chakraborty *et.al*, 2005).

The defense strategies of plants against their pests and pathogens are manifold and include the use of antifungal chemicals. On the other hand, pests and 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. Isolated mycelial walls of many fungi possess potent elicitor activity to induce phytoalexin accumulation in plants (Mansfield, 2000).

Natural products from plants continue to be the subject of novel and straightforward application of pathogen and pest control agents. It is noteworthy that these agents are perhaps most often isolated from plants or associated microorganisms that grow in the humid tropics where the competition to survive and even thrive is intense. Therefore there is the expectation that those compounds may be specially fit to compete. As the development of diverse use of these and succeeding agents continue to evolve, populations, animals and crops will increasingly be protected from a broad spectrum of pest by more selective technologies. These agents may also serve as lead compounds for the development of economically superior agents against pests and pathogens.

Botanical pesticides have received renewed interest because of poor public opinion of synthetics and the increase in organic growing practices. Besides their natural origin, botanicals are valued for their low environmental persistence and alternate mode of action, although their instability and lower efficacy compared to synthetics are barriers to development. Active phytochemicals from new botanicals remain important leads for the development of new synthetic pesticides with clear

commercial advantage. Phytochemicals with insecticidal properties have been isolated and studied from many plant families such as Annonaceae, Araceae, Asteraceae, Guttiferae, Meliaceae and Piperaceae for the development of botanical insecticides an important source of new agro-chemicals.

The ability to induce resistance and utilize it optimally in agriculture depends on fundamental knowledge of biochemical changes and on the specificity and compatibility of the signaling systems that regulate their expression (Chakraborty, 2005). It is generally believed that plants defend themselves against pathogenic fungi by producing fungitoxic substances such as phytoalexins (Purkayastha and Daniel, 1995), pathogenesis related proteins (Castro *et al.*, 2004), oxidized phenols (Arendse *et al.*, 1999) and several other components. Usually a plant responds to a pest or pathogen by mobilizing a complex network of active defence mechanism. The success of the plant in warding-off the pest and pathogenic attack depends upon the coordination among the different defence strategies and the rapidity of the response. But in most cases the role of a single defence component has been reported at a time while working on disease resistance of a host-pathogen system.

In the present investigation attempt was made to elucidate the defense strategies in tea plants against pest (*H. theivora*) and foliar fungal pathogens (*A. alternata* and *C. invisum*) with the following objectives:

- (i) Screening of resistance of tea varieties towards pest and pathogens,
- (ii) immunodetection of fungal pathogens in tea leaves, (iii) determination of the level of phenolics and ascertaining their antifungal activity associated with differential host response to infection against pest and pathogens, (iv) association of defense enzymes (phenyl alanine ammonia lyase, polyphenol oxidase, peroxidase,  $\beta$ -1,3-glucanase and chitinase) in tea varieties triggered by pest and pathogens
- (v) accumulation of pyrocatechol and catechins in tea following infestation by *H. theivora* and inoculation with *A. alternata* (vi) induction of resistance in tea plants against pest and pathogen using bioresources and (vii) cellular localization of defense enzyme in tea tea leaves following induction.