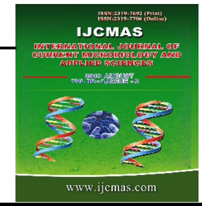

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Original Research Article

Hypersensitive reaction and anatomical changes of young tea leaf (*Camellia sinensis,* clone TV1) during feeding by tea mosquito bug (*Helopeltis theivora* Waterhouse: Hemiptera: Miridae)

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A B S T R A C T

Keywords

Tea; *Helopeltis theivora;* hypersensitive reaction; lesion; *Camellia sinensis;* anatomical change.

This work mainly deals with some aspects of early morphological and structural changes induced during feeding by *Helopeltis theivora* on young tea leaves (*Camellia sinensis*) clone TV1.Infestation of tea leaf by the insect induced hypersensitive reaction which appeared to be similar to that induced by microbial pathogens in plants. The pest is a vascular feeder and the labial stylet pierces protective wax layer, cuticles, epidermal and underlying cells to reach the thin-walled phloem cells and sucks the cell sap. Sap feeding induced hypersensitive reaction resulting formation of typical lesions probably due to release of 'elicitor' with the saliva of the feeding insect. Feeding caused erosion of the wax layer, cuticle and epidermal cells and browning of the cells along the course of the piercing stylet and nearby tissues. Phloem cells, from which the insect sucked sap, did not discolour but collapsed earlier than other cells. Ultrastructural changes evident before collapse of affected cells were collapse of vacuole, degenerative changes in chloroplasts, damage to the cell membrane and cell wall.

Introduction

Tea mosquito bug (*Helopeltis theivora*) is the most destructive insect pest of tea plant in the Northeast region (Hazarika *et al.,* 2009). It feeds and lays eggs on young twigs ('two leaves and a bud') which form the basic raw materials for processed tea of commerce (Rahman *et al.,* 2005). About 80% area of the tea plantation in Northeast India is affected by this pest which reduces productivity upto 10-50% (Gurusubramanium *et al.,* 2007). Average means of shoot infestation were recorded to be 24% and 21% in 2005 and 2006 respectively (Sarmah *et al.,* 2004). *Helopeltis* associated crop damage is as high as 55% in Africa (Rattan, 1992), and 10-50% in Northeast India (Roy *et al.,* 2010a, b). Yield losses from 11-100% has been reported in Asia (Muraleedharan, 1992). The maximal activity of tea mosquito bug is from May-September, coinciding with high temperature and rainfall. Feeding by the adult insect and its nymphs causes loss in quantity and quality of the economically important leaf biomass due to formation of extensive lesions. In addition, oviposition causes stems to develop cracks and over-callousing, causing stunted growth and die-back of stems. The most preferred site of oviposition is a soft part of the shoot below the second leaf and the exposed tissues after a harvest (Bhuyan and Bhattacharyya, 2006). Mirids are vascular feeders and draw sap either from the xylem or phloem, while some are mesophyll feeders. Histological studies show that mirid stylet tracks that terminate in the vascular tissue, in addition to the mesophyll and other tissues (Smith, 1926, Painter, 1928, King and Cook, 1932, Hori, 1971a). But it has not been conclusively demonstrated whether it is a phloem or xylem feeder.

Hypersensitive reactions (HR) in plant and animal host tissues are result of interactions between host and parasite resulting in checking of growth or killing of the invading microbes (Hoglund *et al*., 2005). Similar reactions are also induced by insect-host interactions during sap feeding, but limited studies have been done in this area (Fernandes, 1998). Elicitation of HR has been observed among those insects with a tight association with their host plants, such as galling insects, bark beetles, and wood borers (Fernandes 1990). HR associated with *Helopeltis* herbivory is a prominent example that can cause high yield losses to economically important tea crop. Type of leaf tissue sucked and structural and morphological changes associated with HR due to injuries caused by the insect in young tea leaves are described here.

Materials and Methods

Source of the plants

Fresh twigs of tea variety TV1, susceptible to *H. theivora* (Figure.1), were collected from the New Botanical Garden of Tocklai Experimental Station (TES), Jorhat, and cut ends were immediately immersed in distilled water in small glass containers. Twigs were then transferred to the laboratory and kept in mesh-wired cage maintained at 25°C. Three such cages were set up for the experiment.

Infestation in lab condition

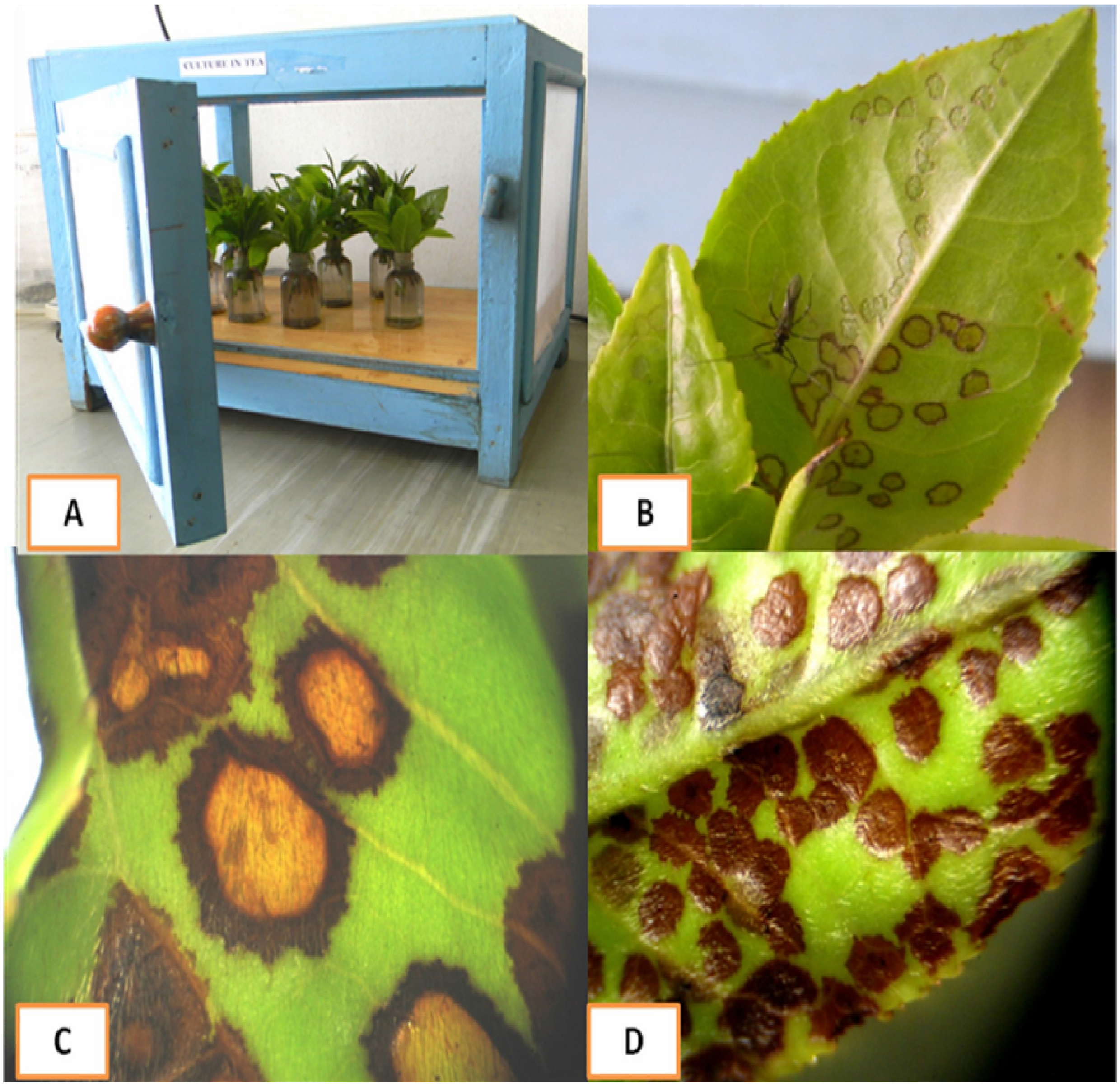
Five adult *H. theivora* obtained from the Entomology Division of TES were introduced into each of the cage and allowed to feed. Continuous observations were made in the initial period of 3-4 hours to trace the development of the lesions caused by feeding. Feeding was continued overnight to record further changes in the morphology of the lesions. Few punctures were made on 3 young leaves with a fine and disinfected stainless needle for comparison with insect infested leaves.

Infested leaf samples were collected at different intervals and fixed in FAA solution for detailed anatomical studies.

Anatomical studies

Stained and unstained transverse sections through the infested parts were prepared, dehydrated passing through alcoholic grades and mounted in DPX for microscopic study. Sections were cleared in 2% KOH prior to staining and dehydration.

Figure.1 Tea-leaf morphology post *Helopeltis*-infestation



A: Experimental set-up for controlled infestation; **B:** Insect puncture marks after 3 h feeding **C:** Magnified image of the typical 'halo' developed after 2-3h feeding; **D:** Abaxial surface of severely infested tea leaf after overnight feeding

Transmission Electron Microscopy (TEM)

The study was undertaken at the Department of Sofisticated and Advanced Instrumentation Facility (SAIF), NEHU, Shillong. Sample preparation was done according to the method followed at TEM facility centre, SAIF, NEHU. Sections were viewed using a JSM-100CX, Jeol Transmission Electron Microscope (JEOL, Tokyo, Japan).

Scanning Electron Microscopy (SEM)

Samples preparation was done according to the protocol described by Dey *et al.*, (1989). The dehydrated and dried leaf pieces were secured horizontally to brass stubs with double-sided sticky tabs, coated with gold in a JFC-1100 (Jeol) ion sputter coater and observed with a JSM-35 CF (Jeol) SEM operated at 15kV.

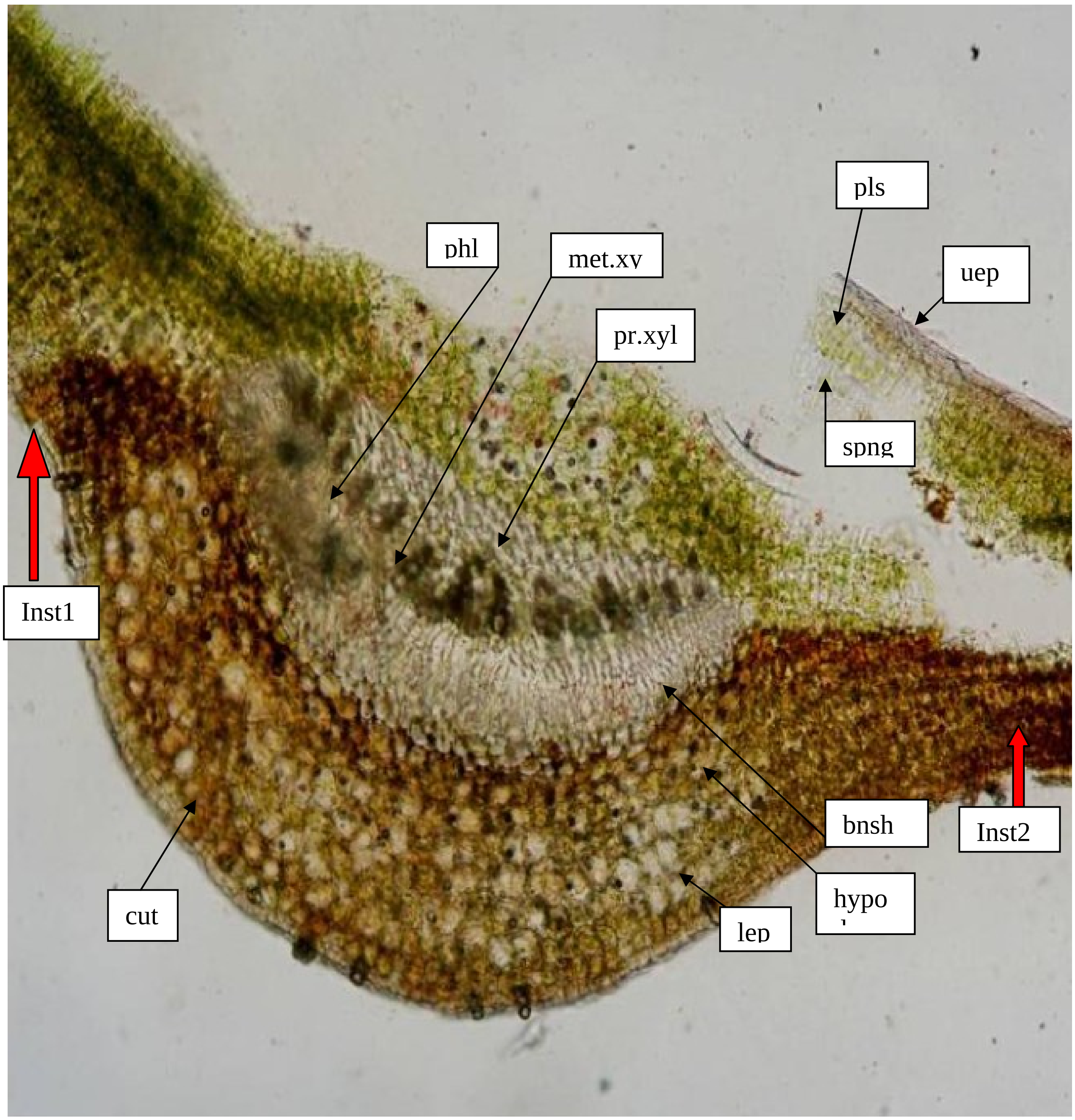
Results and Discussion

Lesion development

After *H. theivora* feeding on the leaf, morphological and histological changes at the point of feeding was rapid and colour change occurred after 10-15 minutes. At the point of entry of the labial stylet, erosion of wax layer, cuticle and the epidermal cells were clearly seen (Figure 2. Inst-1 and 2). In the early phase immediately after feeding, a small translucent spot developed; later all the cells along the course of the labial stylet and upto the bundle sheath layer discoloured quickly accumulating dark brown coloured substances, often with distinct thickening of the cell walls (Figure.2. Inst 1 and 2). Necrosis and disintegration of tissues followed, resulting a typical spreading lesion. Gradually cells, particularly with thicker walls, *viz.*, collenchymas and bundle sheath around the vascular bundles away from the point of infestation also reacted as indicated by their discolouration and cell wall thickening (Figure .2). Interestingly, the phloem tissue just below the bundle sheath and below the point of infestation did not show any discolouration, but showed disintegration and collapse of phloem cells (Figure .2. Inst 1). Xylem tissue remained apparently intact and did not exhibit any abnormality in its integrity and colour at this stage. In a lesion formed in the leaf lamina region away from the midrib, mainly the hypodermal cholenchyma cells became prominently discoloured (Figure 2; Infs 2) and the nearest vascular bundle of the leaf vein/ veinlet showed discolouration. In well developed old lesions, cell necrosis and collapse of tissues were prominent and thickening of the affected parts also occured.

After withdrawal of the stylet by the insect, morphological changes and spread of the leaf lesion continued for considerable time ultimately forming a typical lesion consisting of a central necrotic zone surrounded by a dark coloured ring. On the other hand, no such lesions developed when leaves were punctured with a needle except few necrotic cells around the injured spot. This indicates the initiation of HR due to chemical/s released by the insect at the time of feeding probably for dissolution of wax layers, cuticle and the cell walls of the underlying tissues to facilitate piercing. Kiraly (1980) reported that necrotic response is the result of a disturbance in the balance between oxidative and reductive processes, which results in an excessive oxidation of polyphenolic compounds and a breakdown of cellular

Figure.2 CS of *H. theivora* infested tea leaf (variety TV1) showing sub-epidermal hypersensitive cells around the points of insect injuries (Inst 1 and Inst 2) on the abaxial surface on either side of the midrib. uep: upper epidermis; lep: lower epidermis; hypod: hypodermis; pls: palisade parenchyma; spng: spongy parenchyma; phl: phloem; met.xyl: metaxylem; pr.xyl: protoxylem; bnsh: bundle sheath; inst: insect injury point.



and subcellular structures. The possibility of such biochemical changes in case of *H. theivora* induced HR in young tea leaf is high as such leaf normally contain considerable amount of phenols and polyphenols (Robertson, 1991; Yao, 2005; Obanda *et al*., 1997). Rapid development of the lesions after feeding may be corroborated with presence of such reactive compounds in considerable amounts in young tea leaves.

Ultrastructural changes in leaf surface morphology

Wax forms the outermost protective layer of the tea leaf surfaces above the cuticule. SEM images of the leaf surfaces revealed that the waxy layer had an uneven surface because of irregular deposition of wax (Figure.3, A-D). On the adaxial surface of leaf, the layer was continuous and uniform, while it was discontinuous on the abaxial side because of presence of large number of stomata. During lesion formation after the feeding by *H. theivora*, wax layer on both the surface of lesion showed clear changes in its texture. It became loose and thinner (Fig. 3, B and D) in the lesion area. This may be due to wax degrading enzyme released during feeding and or collapse of the underlying cells at the point of feeding.

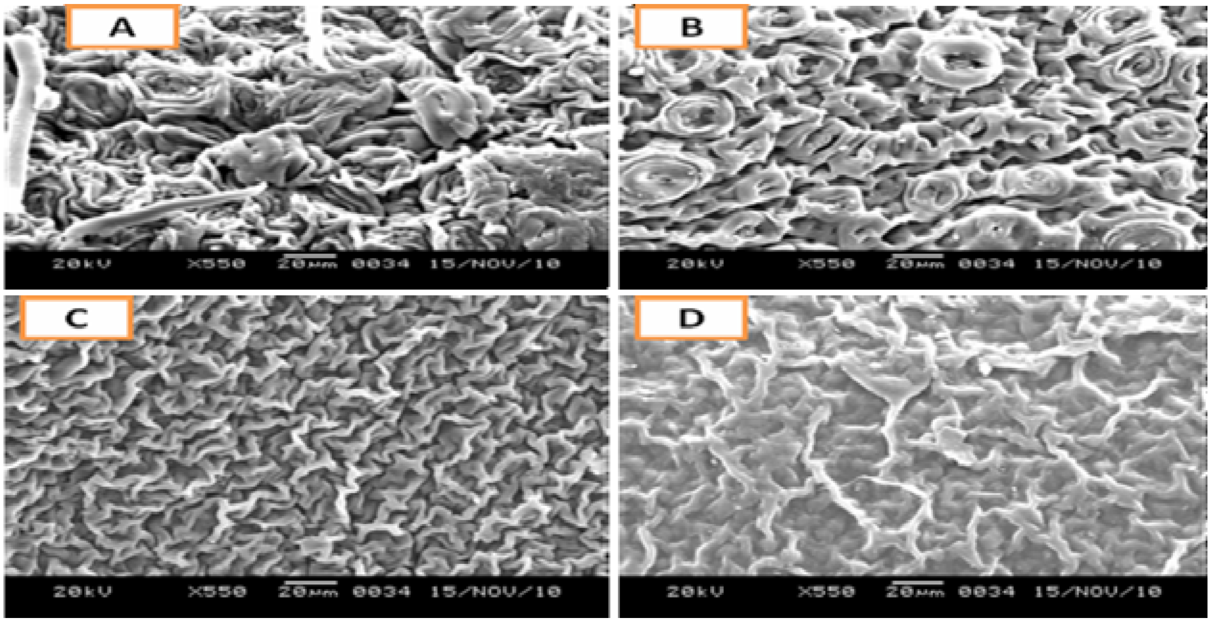
Ultrastructural changes of the affected cells

It is clear from the TEM images that *Helopeltis* induced HR, which resulted in the formation of a typical leaf lesion around the feeding point, induced physical damage to the underlying cells of the mesophyll and other neighboring cells (Fig.4). At the initial stage of lesion formation, rupture of vacuole and changes in the chloroplast morphology is prominent (Figure.4B). Gradually cell wall.

discolouration and cell membrane damage at multiple points set in and the cells started to collapse along with cell organelles (Figure.1D). Necrosis and cell death is normally associated with defense reactions during host-parasite interactions and usually due to inducible defenses including the production of toxic chemicals, pathogen-degrading enzymes, and deliberate cell suicide (Freeman and Beattie, 2008).

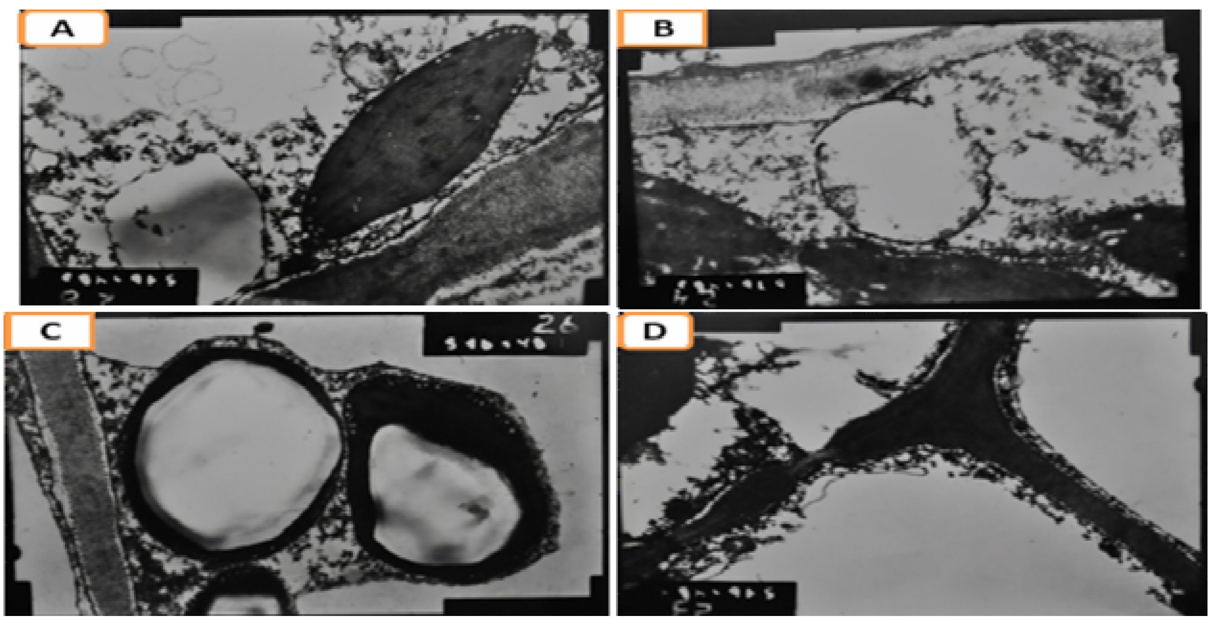
'Mechanical damage caused by insects is not generally considered "true" plant disease although plants have developed surveillance systems designed to recognize insect pests and respond with specific defense mechanisms. Plants can distinguish between general wounding and insect feeding by the presence of elicitors contained in the saliva of chewing insects' (Freeman and Beattie, 2008). This is true in case of *H. theivora* herbivory on tea because lesion formation due to induced HR continued for several hours after completion of sucking by the insect. Moreover mechanical injuries failed to produce any such lesion. Lesions induced by the insect on tea leaf are comparable to those induced by plant pathogens especially on host leaves. Phenolics are a large class of secondary metabolites produced by plants to defend themselves against pathogens and insects (Lattanzio *et al.,* 2006). Discolouration of the cells and formation of a dark brown coloured halo around the dead cells in a lesion indicates the role of an inducible defence mechanism with possible involvement of oxidation of phenolic compounds through polyphenol oxidase (PPO) enzymes, since these compounds are normally found in tea leaves as

Figure.3 SEM images of non-infested and *Helopeltis*-infested tea leaves showing leaf surface ultrastructures



A: Surface morphology of wax layer on abaxial side of non-infested leaf; B: Abaxial surface of infested leaf near the insect injury showing changes in surface texture and morphology; C: Adaxial surface of non-infested leaf with uniform pattern of wax layer; D: Adaxial surface of infested leaf near the lesion showing changes in surface texture and morphology of wax layer

Figure.4 TEM images of non-infested and *Helopeltis*- infested tea leaves



A & C: Non-infested leaf sections showing intact cell membrane, cell wall, vacuole and chloroplast; B & D: Infested leaf sections showing a ruptured vacuole adjacent to degenerating chloroplasts, discoloured and damaged wall and ruptured cell membrane.

important constituents. A detailed biochemical and ultrastructural study involving resistant and susceptible varieties of tea may reveal the actual mechanism of HR in *H. theivora* herbivory on tea.

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