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Cytotoxic effects of *Eupatorium odoratum* on the Ultrastructure of the Midgut Epithelium of late instar larvae of *Orthaga exvinacea* Hampson

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Abstract

A study on the effect of methanolic leaf extract of Eupatorium odoratum on the ultrastructure of the midgut epithelium of sixth instar larvae of Orthaga exvinacea were carried under laboratory condition. Five different concentrations (1%, 2%, 3%, 4% and 5%) of botanicals were prepared. Among the five different concentrations of botanicals tested and by light microscopic studies, the highly effective 4% was selected for the ultrastructural study. The effects of E. odoratum were evidently showed in the ultrastructure of epithelial layer, mainly consisting of excessive vacuolation and elongation of columnar and goblet cells, detachment of epithelial cell from the basement membrane and the presence some secretory vesicles. Severe loss of microvilli were noticed in both inside the goblet cells and apical region of columnar cells. Reduced number of endoplasmic reticulum and shrunken columnar cell nucleus were observed. The active ingredients present in the leaf extract of E. odoratum might be the cause for the destructive changes in the midgut epithelium. Since these changes might lead to serious digestive and metabolic disorders, thereby affecting the growth of the larvae, this botanical can be used for the management of this pest.

Keywords: Methanolic leaf extract, Ultrastructure, *Eupatorium odoratum*, *Orthaga exvinacea.*

Introduction

Orthaga exvinacea, the mango leaf webber is one of the major pests of mango tree. The caterpillars cause serious damage by defoliating the leaves and thereby reducing the crop yield. Distribution of this pest is enormous in different agro-climatic zones and has attained the status of major pest in India[1].

Heavy infestation by this caterpillar directly affects the flowering of the plant and new flush[2] and the infested trees look like burnt and severe infestation might adversely affect flowering[3].

Life cycle of *O. exvinacea* consists of egg, larval stage comprising of six instars, prepupal, pupal and adult stage. The entire life cycle of this species might take 45 to 52 days. The larvae are initially gregarious and feed by scraping the leaf surface. The larvae in late instar stage have the active feeding tendency.

The widely used synthetic insecticides in pest management programme, in order to increase crop yields cause serious health hazards for other non target animals. The residues from these insecticides are dangerous for the consumer and the environment[4].

Plants are rich sources of insecticidal compounds which are found to be highly effective, safe and ecologically acceptable. Biopesticides are regarded to be safe for natural enemies and free from residue problem on the crop and in the environment[5]. Many of the plants possess chemical substances which have antifeedant activity, depressants activity and growth regulatory activity on insects[6].

Many workers have proved the insecticidal activity of *E. odoratum* in pest management programme. Before planting in sweet potato beds, when leaves of *E. odoratum* are mixed with soil, it reduces weevil infestation[7]. Methanolic extracts of *E. odoratum* leaves disrupted oocyte development and vitellogenesis in *Oryctes rhinoceros*[8].

The present study is an attempt to evaluate the toxic effect of *E. odoratum* on the ultrastructural changes in the midgut epithelium of sixth instar larvae of *O. exvinacea* and to prove the potency of this botanical in managing this pest.

Materials and Methods

Culturing of *O. exvinacea:* The pupae and larvae of *O. exvinacea* were collected from the field, reared and maintained in laboratory conditions. The larvae were reared in plastic troughs, fed with fresh mango leaves and kept inside rearing cages. Adult moths emerged were sorted out for their sexes and kept in plastic jars and fed with 50% honey. When the egg hatched, young larvae were fed with fresh tender mango leaves.

Preparation of leaf extracts: Fresh leaves of *E. odoratum* were collected from the field, washed and shade dried. These dried

leaves were ground into fine powder with an electric mixer grinder and sieved through a muslin cloth. 50 gms of leaf powder was extracted using 500 ml methanol in Soxhlet apparatus at 70-80ºC temperature. The extract was allowed to evaporate in a pre-weighed petridish in an oven at 50-60ºC. After complete evaporation of solvent, 10% stock solution was prepared from the weighed extract using methanol. From these stock different desirable concentrations of botanicals were prepared.

Transmission Electron Microscopy: Sixth instar larvae having active feeding tendency were used for the experiment. From the observations on light microscopic studies, on the effect of different concentrations of botanicals (1%, 2%, 3%, 4%, 5%), the highly effective concentration-4% was selected for the ultrastructural studies.

The pre-starved experimental sixth instar larvae were fed with 4% botanical treated mango leaves and the control larvae were fed with methanol treated leaves. After feeding for 48 hours, the larvae were sacrificed to collect midgut tissue and fixed in 3% glutaraldehyde in 0.1M phosphate buffer fixative. Washed and post fixed tissues were dehydrated in the series of ethanol (70%, 90%, 95% and 100%) and propylene oxide and embedded in araldite.

Semi and ultrathin sections were cut using ultra microtome (Leica, Germany). Semithin sections were stained with 1% Toluidine blue and observed and photographed with an Olympus light microscope. Ultrathin sections were stained with Lead citrate and scanned by using a Hitachi H500 TEM.

Results and Discussion

General aspects of histomorphology of the midgut tissue of the normal (control) sixth instar larvae of *O. exvinacea* comprised of a basement membrane with connective tissue and inner to it a layer of epithelial cells and outer to it a muscle layer consisting of inner circular and outer longitudinal muscles which were formed of actin and myosin filaments (Figure-1a,b).

The epithelial cells consisted of regenerative cells, columnar cells, goblet cells and endocrine cells which were not distinctly noticed (Figure-1c). Goblet cells contained numerous microvilli with abundant mitochondria (Figure-1d). Presence of numerous endoplasmic reticulums in columnar cell (Figure-1e) and their apical portion contained numerous microvilli (Figure-1f).

The ultrastructural changes in the larval midgut of *O. exvinacea* treated with *E. odoratum* showed in both muscle and epithelial layer. Elongation of epithelial cells and excessive cytoplasmic vacuolation were noticed in light microscopic studies of semi thin sections (Figure-2a) and in ultrastructural studies, the detachment of epithelial layer from the muscle layer, vacuolated cytoplasmic areas and elongation of both columnar and goblet cells were observed (Figure-2b, c).

Severe loss of microvilli in both inside the goblet cells and in apical region of columnar cells was noticed (Figrue-2d, f). Some secretory vesicles and reduced number of endoplasmic reticulum were showed in the cytoplasm of columnar cell (Figure-2e).

Insect midgut is widely considered as the gut region where the synthesis and secretion of digestive enzymes and the major site for digestion and absorption of nutrients[9].

Active ingredients present in *E. odoratum* might be the cause for the ultrastructural variations in both epithelial and muscle layers of the midgut of *O. exvinacea*. Effect of plant cyclotides on the larval midgut of *Helicoverpa armigera* showed that the cyclotides disrupt the plasma membrane of the epithelial cells forming holes or pores that lead to cell swelling and lysis[10].

In the larval midgut of *S. littoralis* treated with *Azadiracta indica* and *Citrullus colocynthis* extracts, cytoplasmic vacoulation, necrosis and destruction of epithelial cells and their boundaries were reported[11]. Vacuoles result from cell elongation or due to accumulation of fat droplets which dissolves during fixation and dehydration process[12].

Histopathological changes in midgut epithelial cells induced by ingestion of phorbol-type plant isolated compound Jatropherol-I, revealed that the distruction of the epithelial cells caused drastic changes in insect metabolism, especially in protein metabolism affecting the activities of various midgut enzymes[9].

Terra, *et al.*[13] reported that the cellular components that are present in the apical region of the columnar cells are involved in the synthesis of the digestive enzymes, hence the destruction of microvilli in the apical region might be the cause of serious digestive disorder.

Conclusion

In the present study it is suggested that the effect of the active components present in the methanolic leaf extract of *E. odoratum* might be the cause for the ultrastructural alterations in the midgut epithelial cells.

Since these changes might be lead to serious digestive and metabolic disorders, thereby affecting the growth of the larvae, by this prove the potency of this botanical and it can be used for the management of this pest.

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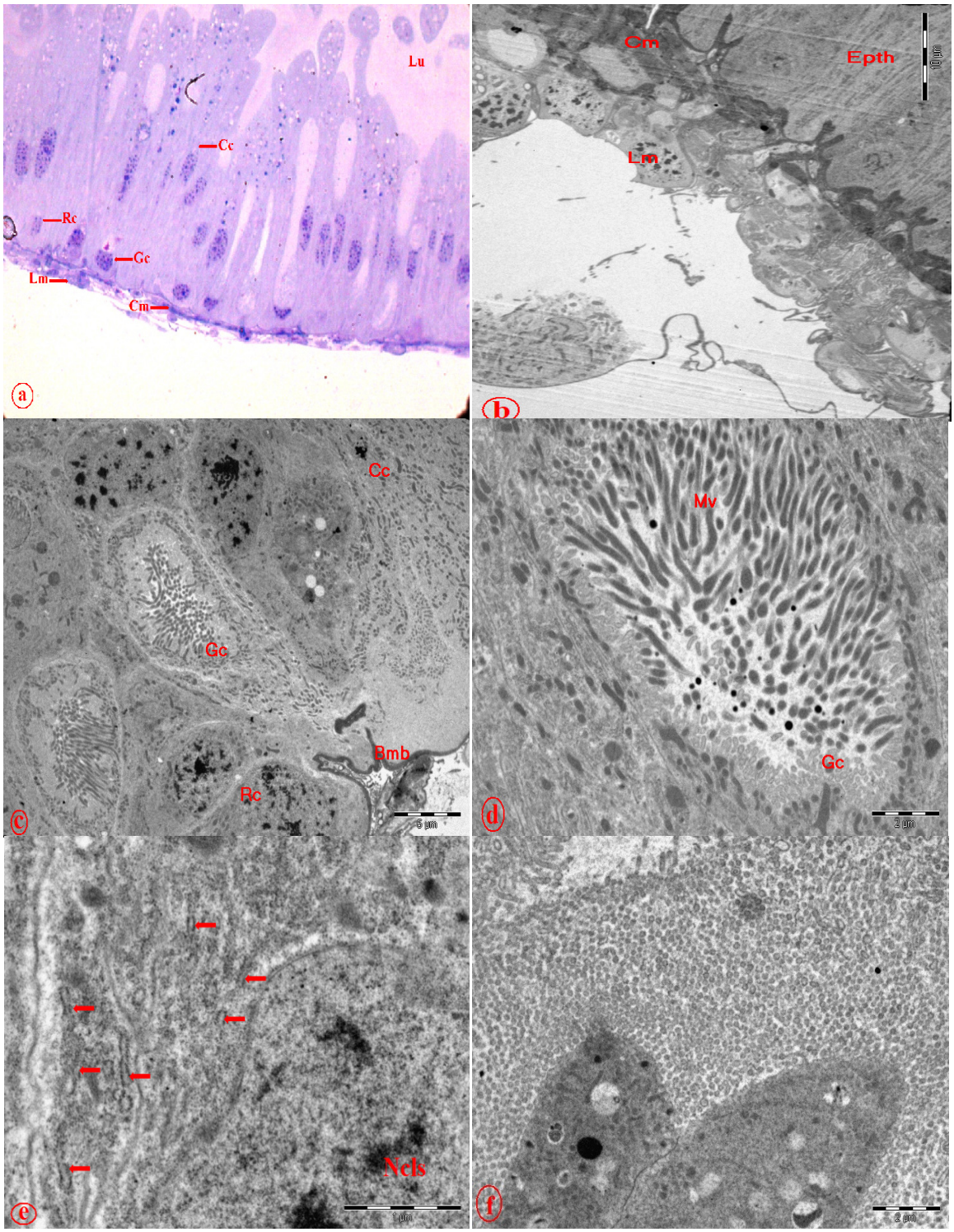

Figure-1

a. General aspects of the midgut tissue of the sixth instar larvae of *O. exvinacea* showing both muscle and epithelial layer (Semi thin section). 1000X b. Ultrastructural aspects of longitudinal muscle layer (Lm); Circular muscle layer (Cm) 1900X. c. Ultrastructural aspects of different epithelial cells 2900X.d. Ultrastructure of untreated goblet cells (Gc) having numerous microvilli (Mv) 6800X.e. Presence of numerous endoplasmic reticulum in columnar cell 23000X. f. The apical portion of columnar cells with microvilli 6800X. Abbreviations:-Bmb- Basement membrane, Rc- Regenerative cell, Cc- Columnar cell, Gc- Goblet cell, Lumen and Mv- Microvilli, Epth- Epithelial cell layer.

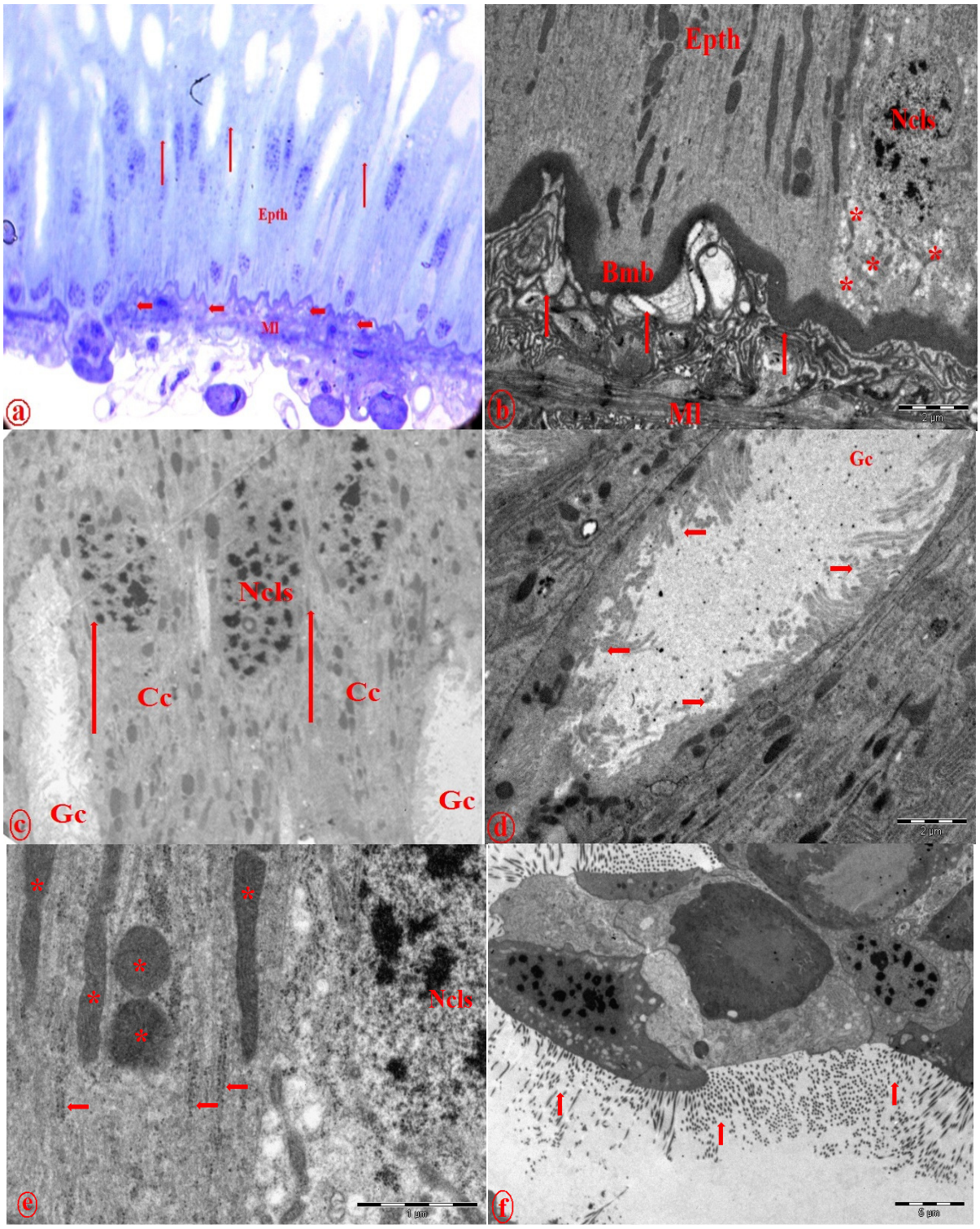

Figure-2

a. Semi-thin section of the midgut tissue treated with *E. odoratum* showing the elongation of epithelial cells (upward arrows); detachment of epithelial layer from the muscle layer (arrows) 1000X. b. Showing ultrastructural aspects on the excessive vacuolation (asterisks) and detachment of epithelial layer from the muscle layer (arrows) 6800X. c. Showing elongation of both columnar and goblet cells (upward arrows) 1900X. d. Loss of microvilli in inside the goblet cells (asterisks) 6800X. e. Showing Secretory vesicles (asterisks) and reduced number of endoplasmic reticulum (arrows) 23000X. f. Loss of microvilli in apical region of columnar cells (arrows) 2900X.

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