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HISTOARCHITECHTURAL ALTERATIONS IN THE MIDGUT TISSUES OF FOURTH INSTAR LARVAE OF GRAMPOD BORER *HELICOVERPA ARMIGERA (HUB)* FED WITH LEAF EXTRACT OF PLANT *LANTANA CAMARA (L)*

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ABSTRACT

The gram pod borer *Helicoverpa armigera(Hubner)* (Lepidoptera: Noctuidae) is an important notorious pest of more than 100 cultivated and wild plants all over the world. Switching off of pest control strategies from chemical to biological using microbial and herbal biopesticides are becoming more viable due to their sustainability in the environment. In the present study, ethanolic leaf extracts were fed to fifth instar larvae and the process of infections were assessed on the basis of morphological changes followed by alteration in the midgut tissues. The treated larvae showed sluggishness,cessation of feeding and gradual shrinking of larval body leading to death at last.The midgut tissues were completely distrophied with thin and feeble architecture.Columnar epithelial cells became shrunken with atrophied nuclei.Peritrophic membrane became merged with midgut tissues allowing tissues ooze to mingle with the lumen.Ultimately, only some cytoplasmic residues were left in the cavity.Thus, plant leaf showed its potential in controlling notorious pest by exerting its effect on the feeding process of the insects.

KEY WORDS

Helicoverpa armigera(Hub),*Lantana camara*(L),leaf extract,columnar epithelium,goblet cells,regenerative cells,peritrophic membrane

INTRODUCTION

The American bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera : Noctuidae) is a well known polyphagous pest distributed throughout the tropical and subtropical parts of the world. It is widespread in India, particularly in Rajasthan, where it exists as an endemic pest. It is a very destructive pest occurring on cotton tomato, okra, chickpea, sorghum and many other crops, causing substantial crop losses every year[23,15,29]*Helicoverpa armigera* (Hubner) is also characterized by its high mobility and fecundity and it has shown great capacity to develop resistance to synthetic insecticides used in its management[10,11] It has been recorded on more than 100 cultivated and wild host plants[10]. Outside Europe, *Helicoverpa armigera* (Hubner) is an important pest of cotton and many other crops in many countries[7,8]. Of late an annual crop loss due to *Helicoverpa armigera* (Hubner) in India has been estimated at around Rs. 200 crores per annum[1]. However, resistance to all commercially available insecticides has been detected in *Helicoverpa armigera* (Hubner). The increasing emergence of resistance problems means that there is an urgent need for the development of management. Strategies, which are less dependent on chemical insecticides or less conducive to the development of resistance to the control measures are more successful. Plants are endowed with a potential to produce a wide spectrum of allelo-chemicals[22]. Plant derived pesticides are eco-friendly, non-toxic to non target organisms, non persistent in nature, besides they do not promote drug resistance[18] .Plant derived photochemical has been widely used in the management of agricultural pest[2]. . In the field, their rapid degradation and action as stomach poisons make them more selective in some instances for plant-feeding pest insects and less harmful to beneficial insects[10].

Taking the above facts into consideration,in the present investigation a new weed plant *Lantana camara(L*)was taken against the pest *Helicoverpa armigera*(Hub).*Lantana camara* (L.)

(Verbanaceae), commonly known as wild or red sage is the most widespread species of this genus and regarded both as a notorious weed and a popular ornamental garden plant[30]. However, it is listed as one of the important medicinal plants of the world[32] . An investigation on the insecticidal, antiovipositional and antifeedant activity action of *Lantana camara* (L.) against *Callosobruchus chinensis* (Coleoptera : Bruchidae) was done and the plant proved very effective[33]

Ethanolic extracts of leaves were prepared and given to fifth instar larvae of *Helicoverpa armigera*(Hub) and the toxicity of the plants were assessed on the basis of morphogenetic changes as well as effect on mid gut tissues.

MATERIALS AND METHOD

Culture of *Helicoverpa armigera* (Hubner) was maintained in the laboratory in sterilized plastic containers containing crushed artificial diet[17]. The plastic containers were pierced for

proper aeration and the culture was maintained at 28^0 C ± 2^0 C and70 ±75 percent relative humidity.

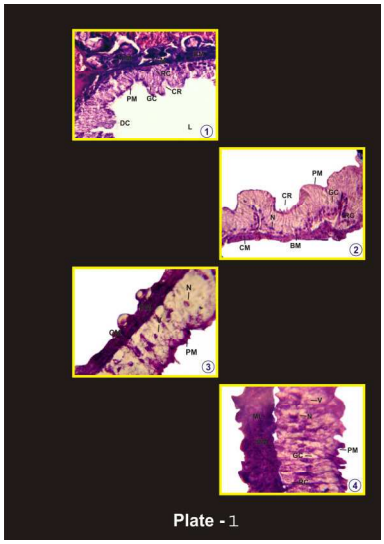
Leaf extract preparation : Fresh leaves of the weed plant *Lantana camara* (L.) were collected, washed, shade dried and then powered. Extracts were prepared by soxhlet apparatus in the ratio of 1:10 that is 10grams of leaf powder and 100ml of solvent (ethanol). After 8hrs. of continuous extraction the final extracts were kept as stock solution (100 percent)[4].

The treatment method used was feeding method (mixed with food). For experiment , 1gms of artificial diet was mixed with different doses of the leaf extract(0. 1, 0. 5, 0. 75 and 1. 0 ml). The diet was left for half an hour for the ethanol to get evaporated. Since there is a severe cannibalism, so 10 larvae per replica per dose in each case were released individually in separate containers i.e., each container contained one larva with 1 gram of food mixed with desired dose of extracts.Control set were run with ethanol mixed food . After 24 hours, fresh diet was provided to the insects. After screening, the effects of botanical on mid gut tissues were studied with highst applied dose by histopathological techniques. Histopathological work was carried out by standard methods of microtomy. Whole body section of untreated and treated (leaf extract) fourth instar larvae were taken to observe the effect of leaf extract on midgut tissues.. Fifth instar larvae were not taken into account as feeding became slow and larvae started preparing for pupation.

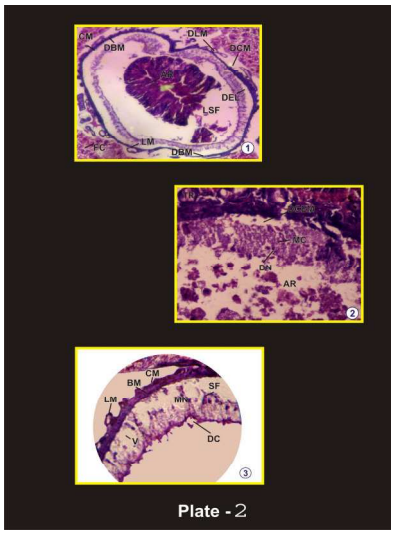
RESULT

Normal histoarchitecture of midgut (Plate-1,Fig-1,2,3&4)

It is one of the the main organ of the insects body as far as the effect of any chemical is concerned. In the present investigation, midgut of the fourth instar larva of *Helicoverpa armigera* (Hubner) revealed various histoarchitectural arrangements



Normal histoarchitecture of midgut



Histoarchitecture of leaf extract treated midgut

The normal histoarchitecture of midgut area of fourth instar larvae of *Helicoverpa armigera* (Hub)(Plate-1,fig-1)showed central rounded part surrounded by thick layers of circular and

longitudinal layers(CM&LM).It is the mid gut area. The midgut wall reveales three layers, the inner columnar epithelium(CE), the median basement membrane(BM) and the outer muscular coat with the circular and longitudinal muscles. (Snodgrass, 1956). (Plate-1,Fig. 1 and 2).Columnar epithelial cells are seen with nuclei at middle or distal parts of cell bodies. In section, they were visible in regular lines near the base of the cells. (Plate-1,Fig. 3)The basal layer of epithelial cells rests on basement membrane towards periphery and the tip of cells faces lumen(L) filled with alimentary residuals. (Plate-1, Fig. 2,3 and 4) .Appearance of epithelium mostly depends according to the state of digestive process. Cytoplasm appears granular with large nucleus occupying central or distal position. In section, they form fairly even rows or lines following the inner layer of epithelium. Cells contain homozygous cytoplasm, regular and intact cell boundaries. (Plate-1,Fig. 2)In addition to tall and columnar cells, which are most abundant, large spongy cells are usually seen in groups in between the bases of columnar cells and called as Goblet cells(GC) or digestive cells taking active part in process of secretion with their opening at their inner ends projecting into the gut lumen. (Plate-1,Fig. 4).Smaller basal cells arefound in small groups in form of patches at the bases of epithelial cells, which are regenerative cells.(RC) These cells function to replace digestive cells when exhausted or at the time of ecdysis. (Plate-1,Fig. 4).Lumen of midgut is lined by peritrophic membrane(PM) protecting the tissues of midgut from hard food particles.(Plate-1,Fig3&4)

Histoarchitecture of leaf extract treated midgut (Plate-2,Fig-1,2,3)

Histopathological investigations explained and discussed the efficacy of leaf extract treatment of plant on the insect mid gut tissues.Leaf treated larvae of *Helicoverpa armigera* (Hubner) revealed complete degeneration and dissolution of midgut layers with columnar epithelium detached completely on all the areas leaving an irregular space all around beneath the muscular layer. (Plate2-Fig. 1)No demarcation between the basement membrane and muscular layers could be seen. The cell dimensions as well as the architecture were reduced significantly. (Plate-2,Fig-2)Columnar cell nuclei migrated towards apex and finally disintegrated. (Plate-2,Fig. 2&3).No demarcation of goblet and regenerative cells were seen. Coherent sheet of columnar epithelial layer became loose, damaged, shrinked and distorted completely. (Fig. 2 and 3)At later stages, cells mingled with each other, nuclei disappeared or diminished, cytoplasmic residues scattered with no distinct cell boundaries. (Plate-2,Fig-3).Peritrophic membrane almost lost their existence.Large space formation and mingling of nuclei were prominent in leaf treatment(Plate-2,fig-3)

DISCUSSION

The first attack of the plants extracts were probably on the midgut tissues. Midgut is an organ where digestion, absorption and assimilation of food take place[3,34]. The insects digestive system is one of the main physiochemical barriers against many toxins and pathogenic agents. Hence, the toxins or insecticides that are taken by insects during feeding, if cross these barriers, they are ought to damage the cellular architecture. *Lantana camara* (L.) contains lantadene A and lantdene B which are triterpenoides and exhibit insecticidal properties [24,30]. Another compound camaric acid is also isolated, which is a diverse chemical that acts as larvicidal against mosquitoes[5]. A variety of terpenoides, flavonoids, saponins have been isolated from leaf and flower extracts of *Lantana camara* (L.) and it is observed that phytochemicals of plants might be the reason of

larvicidal activity against mosquitoes[14,27]. It is very interesting to observe that the external pathological symptoms that were observed during screening that was atrophied larvae, weak and shrunken body, delicate larval skin, cessation of feeding, oozing out of liquid from the hind end of the larvae, all clearly revealed that midgut stopped functioning due to the damage in its cellular architecture.Our results are in agreement with the work of many workers,[31,32] where they observed external and cytological symptoms coincided when *Spodoptera liituralis* larvae were fed orally with insecticides. As the external toxicity symptoms advanced there was a definite synchronization between the external toxicity symptoms and pathological effect upon the midgut epithelia.

Further, the plant acts as stomach poison and is toxic as well as antifeedent[26]. Plants have also been reported to synthesize precocenes a juvenile hormone analogue compounds[7.] These reports made the platform to think that after ingesting the food mixed with the extracts, the toxins of the plant extracts acted similar to those of the insecticides and started damaging various layers once being absorbed by midgut.Once entered in body, extract acts on peritrophic membrane that acts as a barrier and selectively permeable membrane surrounding the lumen of the gut. In leaf treated insect the peritrophic membrane became very feeble and almost disappeared and in flower treated insects , this membrane could not be seen due to excessive cell elongation, and blebbing of materials in the lumen from the tip of the columnar cells. The Peritrophic membrane is made up of chitin[9] and chitin inhibitory nature of plants like Azadirarchtin is well documented[26]. It protects the smooth intestinal epithelium from hard food particles and to some degree from pathogen. It is an acellular sheath that protects the food particles entering directly into the midgut[22]. The complete abrasion of this membrane is an indication of toxins acting as a chitin inhibitor as well as barrier of toxicants and pathogens. The plant extracts have acted on such properties of peritrophic membrane that might have resulted in complete abrasion of this membrane causing the food along with the toxicants to enter into the area of the midgut epithelial layer. Such functions of peritrophic membrane is well documented in literature[6,22,26]. Presence of lectins in plants shows resistance in plants towards insects. Lectin binding affects the properties of peritrophic membrane[27].

Once the peritrophic membrane is abrased and destroyed the food along with the toxicants got an easy entry into the mid gut. Since, the columnar epithelial cells are the first one to encounter the intoxicated food the effects of the toxicants were quiet logical. In our investigation, columnar epithelial cells revealed maximum degeneration. In flower treated insects, the cells became particularly thin and elongated with fused boundaries and smaller nuclei and showing blebbing of the contents of cells in to the lumen. At later stages, columnar epithelial cells appeared to be exploded with scattered debris of cellular inclusions.

Our observations are in agreement with many studies[11,12,13], where they observed similar blebbing and exploded columnar epithelial cells in honeybee fed with insecticides malathion, deltamethrin and thermomethoxane.

If we see the effects of leaf extract on the midgut tissues, it revealed a small degenerated and dystrophied epithelial cells with cell membrane decayed completely and detaching from the basement membrane. It created a space between the two and later on large vacuolization in the cells itself. Similar effect of Azadirachtin on midgut cells of *Schistcerca gregaria* and *Locusta migratoria* have also been found[22,25]. The observed various pathological changes were slow necrosis, reduction in size, and number and damaged circular and

longitudinal muscles. At higher dose full destruction of midgut cells with cessation of feeding was also observed during our investigation. Since tissues were taken at highest dose level (1. 0ml) shrinkage in epithelial cells with leaf treated extract was in agreement with the histopathological changes due to insecticide treatment in *Periplanata americana* treated with carbaryl (5. 0x $10^{-2)}$), where shrinkage of columnar cells along with nuclei and evacuation of nuclei were observed[13,14]

The insects possessed detoxifying enzymes that protect them from toxicants[35]. As the concentrations of extracts increased the detoxifying capacity, the cells lost their capacity to recover; thereby resulted in accumulation of dead cells. It was further supported by loss of regenerating cells. In our investigation, continuous elongation of epithelial cells, fading of the cell boundaries, shrinkage of the cytoplasm and degeration of cells in the DDT treated mid gut of *Spodoptera litturalis*.were also observed.[32] The weak and fragile peritrophic membrane got abrased and allowed the entry of toxins into the midgut thereby damaging the cells of epithelium. Since the whole layer of columnar epithelial cells showed damage, it was quite obvious that regenerative cells might have lost their function and the damaged atrophied cells were no longer able to show any secretory activities. It was evident by loss of identification of goblet cells. They could not be seen in leaf treated insects and visible somewhere as small atrophied cells in flower treated insects. The gypsy moth when fed in *Quercus cerris*, and *Robinia pseudoacacia* leaves, the peritrophic membrane became thin and delicate, the goblet cells reduced and columnar epithelium cells became thin and vacuolated[21,22,23].

CONCLUSION

In our investigation, we found that basically the extracts were acting as stomach poison in addition to some antifeedant activities. Although there was not complete cessation of feeding, the insects started showing changes, one to two days after treatment . The alternate strategies emerged under the heading Integrated Pest Management (IPM) where a number of less hazardous chemicals as well as nontoxic biological products were recommended and tried. Hence, if these botanicals are included as routine pest control biopesticides for which they are capable, their commercial production will reduce the cost too. Further, the results, if analysed critically, give a clear indication of bright future prospects of botanicals to be included in IPM.

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