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ORIGINAL ARTICLE

STUDIES ON THE IMPACT OF AZADIRACHTIN ON THE DIGESTIVE TISSUES OF THE ADULT *MYLABRIS PUSTULATA* THUNBERG (COLEOPTERA: MELOIDAE)

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ABSTRACT

The blister beetle *Mylabris pustulata* (Thenberg) a harmful crop pest, voracious feeding to flowers and to cause the Sevier damage of different agricultural plants. In this study aims to observed, the histopathological changes in selected tissues of digestive system of *Mylabris pustulata* Thenberg, when treated into phytopesticide, vijai neem. The neem, Azadirachtin is widely used in Indian agriculture. The remarkable changes were observed in fore and midgut , such as disintegration of nucleus, nuclear pycnosis, cytoplasmic vacuolization, detachment of muscle layers etc.,

.**Keywords** MDPT, IR, Microanalyser, Proline, Potentiometric technique .

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1.INTRODUCTION

The alimentary canal of an insect is simply a tube passing through the body that provides the special internal environment for food to be mechanically and chemically disintegrated and brought to the vicinity of absorbent cells.

The comprehensive account on structure and physiology of digestive system of insects has been meticulously complied by specialized chapmen (1985). In accordance with highly specialized feeding habit the feeding apparatus and alimentary canal are greatly modified both in the larvae and adult (Srivastava and Bogawats, 1968). Histologically foregut is formed of cuboidal epithelium lined by chitinous intima (Muraleedharan, 1983). The oesophageal invagination acts as an oesophageal valve for preventing regurgitation of food materials and is found in honey bees.

The midgut of insect comprises the longest and functionally most important part of the digestive tract, dealing primarily with the digestion of food stuffs and the absorption of nutrients. The midgut is made up of columnar or cuboidal cells supported by a basement membrane and covered by longitudinal and circular muscles. The intima of the hind gut is more permeable than that of the foregut (Chapman, 1985). Rectal lumen of Dysdercus cingulatus is filled with clear transparent watery fluid. (Muraleedharan, 1983).

Owing to the active cell proliferation and profuse secretary activity, the midgut in beetles forms a proficient region of significance in the digestive tract. The histology of coleoptera is most significantly studied and almost remain neglected during recent years. Studies on morphological aspects of the alimentary canal are confined to a few orders of insects like orthoptera (Balakrishnan, 1987), Diptera (Rohatgi, 1985),Hymenoptera (Shyamalanath and Forbes, 1980), and Hemiptera (Krishnakumari, 1986).

Histologically foregut is formed of cuboidal epithelium lined by chitinous intima (Muraleedharan, 1983). The oesophageal invagination acts as an oesophageal valve for preventing regurgitation of food materials and is found in honey bees.

The application of pesticides and insecticides to control insect pests requires a thorough knowledge of the biology of insect pests and their histomorphology of various advanced studies have shown that administration of insecticides affect the cells of various systems and organs insects. On treating the animal with insecticide drastic changes are seen in the alimentary canal.

It is very essential to study the histological changes of midgut epithelium as the digestive activity is mainly taking place there. Further it is also essential to study the histological changes of foregut and hindgut because these regions are concerned with storage, mastication and reabsorption food stuffs respectively. A review of literature reveals that the effects of different types of insecticides were studied on the tissues of insects in different systems and changes were noted in the various types of tissues and cells.

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Neem an Azadirachtin in Insect control the complexity of the molecular structure of azadirachtin precluded its synthesis for pesticide use. Extracts of neem seeds containing azadirachtin together with several structurally related molecules have formed the basis of neem usage in insect control (Isman 1997). Future approaches may also include the production of azadirachtin for insect control by in vitro tissue cultures of neem (Allan et al. 1999). Neem insecticides are effective mainly as insect growth regulates and sterilants, against a broad spectrum of pest insects. Senthilnathan et al. (2005) have studied the toxicity and physiological effect of neem limonoids on Cnaphalocrocis medinalis the rice leaf folder.

From the review of literature it is revealed that the histopathology of the alimentary canal of Meloidae insects has not been extensively studied. So further studies in the histopathological aspects of the digestive system of other insects in the family should be needed. The present investigations have been carried out on *Mylabris pustulata* with emphasis on the morphology, histology and histopathology of selected regions of alimentary canal.

2.MATERIAL AND METHODS

Rearing techniques

The beetles were collected from the field. They were reared in wooden cages measuring about 30 x 33 x 45 cm dimensions at laboratory temperature 29° ± 2°C. The floor of the cage was covered with fine sands; moderately ministered with water daily in order to maintain the humidity inside the cage. Top of cage was covered with glass to provide the sufficient light. Sides of the cage were fabricated with wire meshes. The insects were fed daily with its host plant flowers and cotton soaked in water was provided. Proper cleaning was made on alternative days to remove the excretory material and waters.

Mode of insecticide treatment

For the present study, the commercial Neem pesticide (Vijay Neem, 0.03% EC), Azadirachtin was administrated. The 26 gauge sterilized insulin needle was used for the purpose of injection. Adult insects of *M. pustulata* were collected from the rearing cage and were injected with the median lethal dose (LD_{50} for 24 hours was found out as 200ppm) of 0.001 ml of Azadirachtin through the intersegmental region on the ventral side of the abdomen. The injection site was sealed with low melting wax to prevent loss of haemolymph. The control insects were injected with 0.001 ml of distilled water.

Histological Techniques

Normal healthy insects of both sexes three days after adult emergence were used for the present investigations. Insects under ether anaesthesia were immersed in insect ringer solution and dissected under a streoscopic microscope. Alimentary canal was dissected out intact within the minimum possible time. The foregut, midgut and hindgut tissues were cut into separate pieces and were fixed in Bouin's fluid overnight. Subsequently, the tissues were washed and dehydrated using ascending series of alcohol. To facilitate orientation of tissues during embedding, the digestive tissues were stained with 70% eosin. After dehydration the tissues were cleared in acetone and xylol. Then they were treated with hot and cold infiltrations and finally embedded in molten paraffin at 600c. Blocks were prepared in paraffin and sections were cut at 6 ~ thickness using a rotary microtome (Spencer 820). After spreading, deparaffinization and hydrated in descending order of alcohol, sections were stained and counter stained with Heidenhain's Iron haemetoxylin and eosin respectively. Sections were mounted in DPX for subsequent light microscopic studies.

3.RESULTS

The alimentary canal is straight and slightly convoluted one in the adult blister beetles *Mylabris pustulata*. The alimentary canal is separated into three regions, the anterior stomodaeum or foregut, middle mesenteron or midgut and posterior proctodaeum or hindgut. A salivary gland is absent and three pairs of malphigion tubules are evident in the adult commonly.

Histological Observation

Since the foregut is known to be of ectodermal origin it is lined with a layer of a cuticle (intima) which shed at each moult in the same way as the rest of the cuticle. The intima of the foregut is mostly musclerotized flexible, two major types of spines (Sp) can be noticed.

The first is large backwardly directed spines (LSP) on the walls of the cibarial cavity which extends back into the pharynx (PH) and the ooesophagus (OES) arranged in 5-8cuticule folds which are armed with rows of well developed spines. The second is the small spines (SSP) following directly the large spine (LSP) which are concentrated mainly on the crop (CP) area, and scanty on the proventriculus (PV) area can be seen.

The cuticle layers (the intima) covered these folds and the whole foregut. This layer of the epithelium laying longitudinally beneath these folds. The most distinctive parts in this area are 3-5layers of the circular muscles (CM) which are well developed and continue all round the gut. The external longitudinal muscles (Ex.LM) are absent but the internal longitudinal muscles (ILM), have been embedded beneath the sphincter circular muscles (CM). The folds are supported by the circular muscles together with the connective tissues which give the necessary flexibility during contraction.

The epithelial cells are characterized by large spherical nuclei situated basally and a bulk of cytoplasm containing granular inclusions. The epithelium of the crop of mostly unfolds and extremely thin due to which lumen of the crop enlarges greatly and it is capable of storing a large amount of food material. The epithelial cells are mostly regressed, containing small nuclei and little amount cytoplasm (Plate 1 ; fig A,B,C).

The proventriculus is lined with a simple cuboid epithelium, the proximal regions has wide and short folds, some of which have short and thick spines whereas others have sclerotized plates . The distal regions projects towards the midgut to give rise the stomodeal valve and thickness of the cuboids epithelium increases, the intima disappear. The foregut opens into the midgut through a distinct stomodeal valve.

In the adult, the wall of the midgut is composed of the outer irregular layer of longitudinal muscles, middle distinct layer of epithelium. The wall is externally enclosed into the peritoneal sheath. The circular muscles layer is comparatively thinner than that of stomodeal and it is well developed in the adult. The midgut epithelium is greatly developed and rests upon a basement membrane. It is thin and slightly folded in the anterior region but becomes thick and greatly increased in length. The peritrophic membrane is well evident in the lumen of the midgut. (Plate 1; Fig D & E).

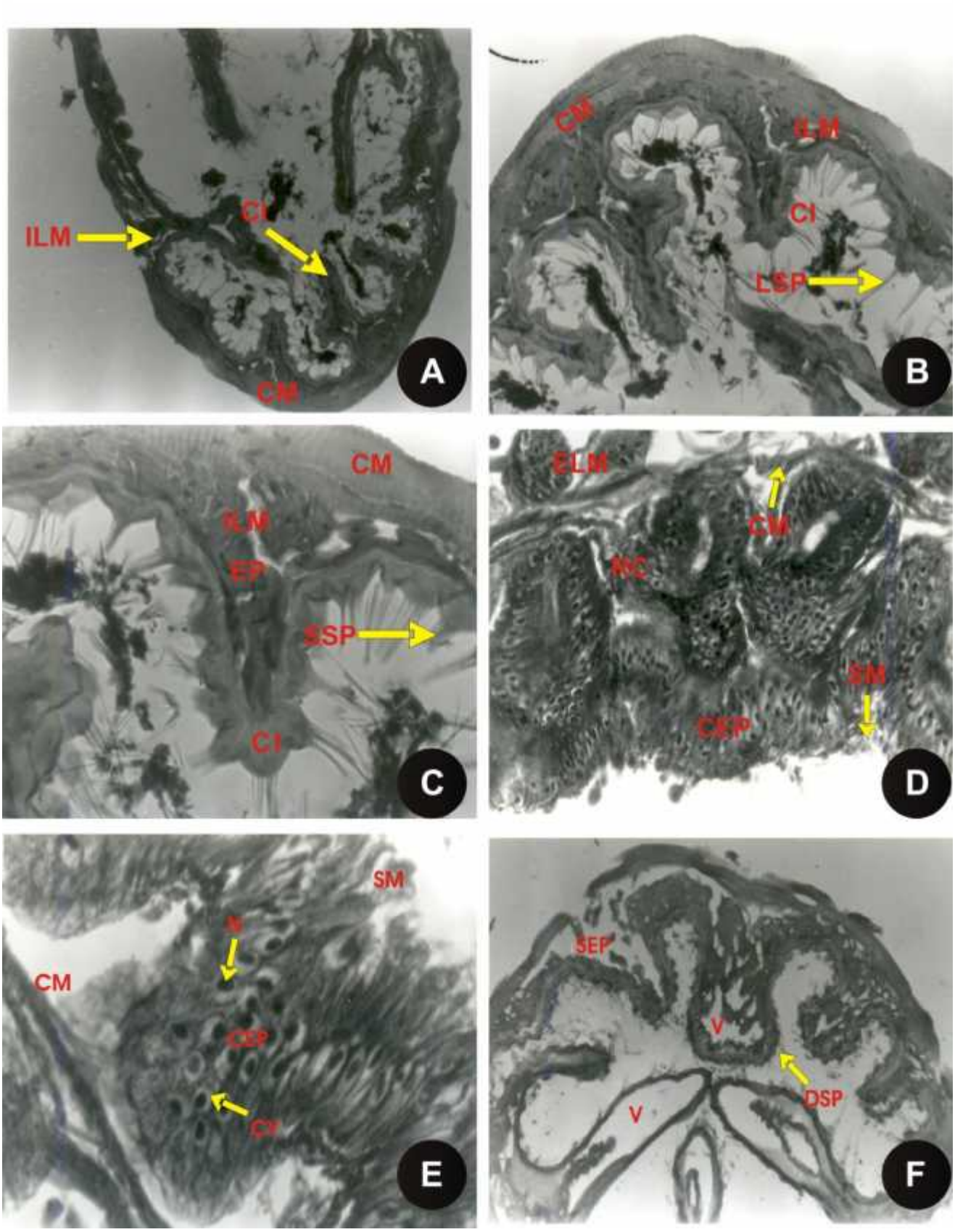

Plate 1-Fig A – C ; control foregut ; D and E control midgut; Fig F treated foregut of *M.pustulata*-CM- Circular muscle;CI-Cuticular Intima;CEP-Columnar Epithelium;CY-Cytoplasm; ELM-External longitudinal muscle;DSP-Disintegration of spines;ILM-Internal longitudinal muscle;LSP-Large spines;N-Nuclei;RC-Regenerative cells;SM secretory materials;SSP-Small spines;V-Vacuoles

PLATE 1

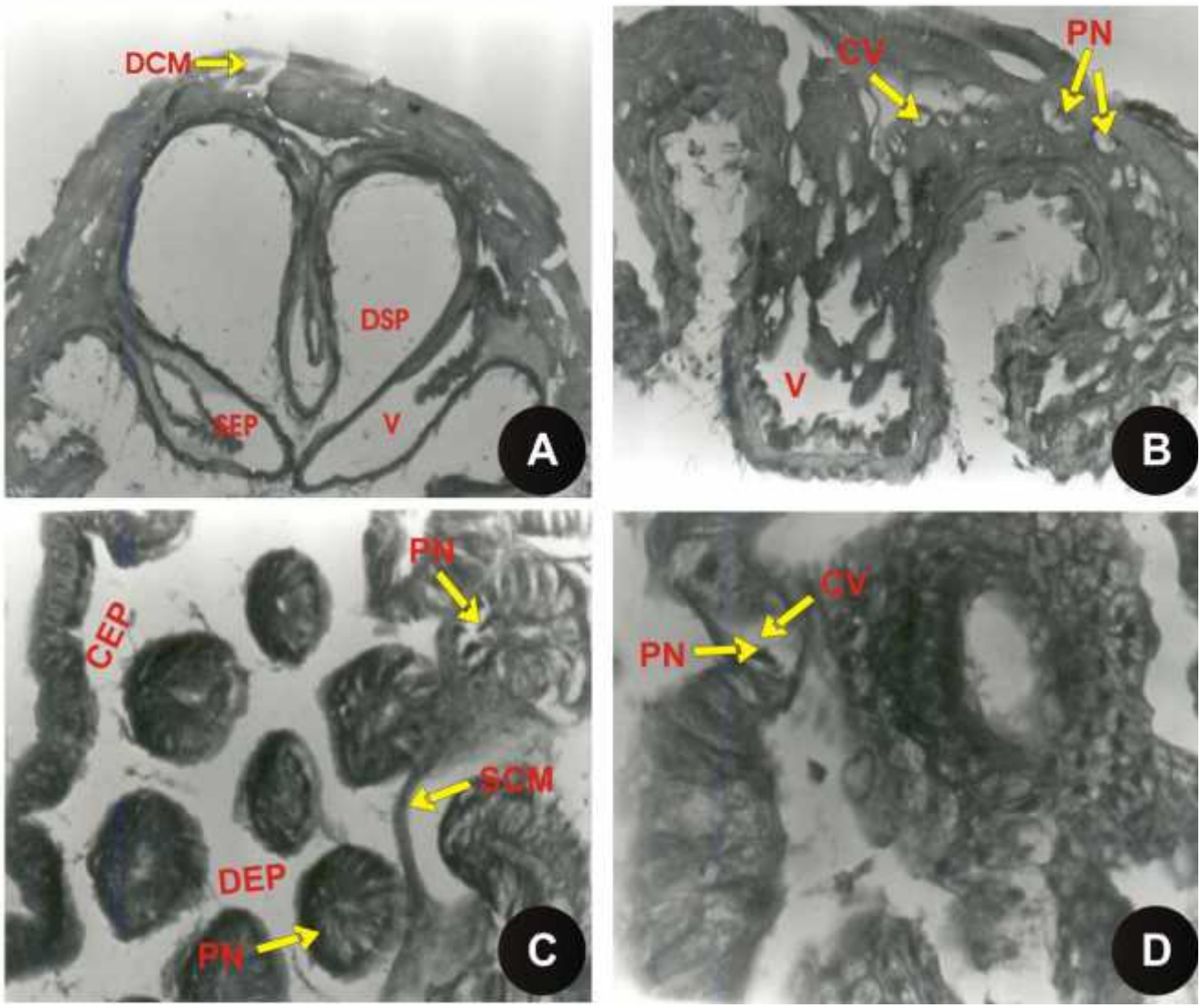

Plate 2; Fig A and B treated foregut; C and D treated midgut of *M.pustulata*.
CEP-Clumped epithelium;CV-Cytoplasmic vacuolization;DSP-Disintegrated spines;DCM-Detached circular muscle;PN-Pycnotic nuclei;SCM-Shrunken circular muscle;SEM-Shrunken epithelium;V-Vacuole

Histopathological Observation

Different histopathological changes were observed in the alimentary canal of *M.pustulata* after 24 hr of treatment with the insecticide Azadirachtin, various changes were studied separately in foregut, midgut and hindgut.

The fore gut signs of shrinkage in the epithelial cells increases considerably as evident by the space between epithelial cells and intima, cytoplasmic vacuolization increases, due to the condensation of the nuclei some epithelial cells take dark stain, the basement membrane looses contact with the muscular layer, Intima gives a discontinues appearance due to breakage, some epithelial cells nucleus seen in the pycnotic condition,the spines were damaged. Plate 1 Fig F); Plate 2 ;Fig A & B).

The midgut epithelium, nucleus and cytoplasm of epithelial cells seem to have been affected (Plate 2 Fig C &D),like some vacuolization in epithelial cytoplasm, epithelial cells in pycnotic condition as evident by the dark stainig of the cells, some epithelial cells seen without nucleus, the apical portion of the epithelial cell, the microvilli were damaged and muscular layer were detached from each other.

The shape of the epithelial cell varied considerably and the outlines of some were indefinite. The tips of most cells were greatly elongate, almost completely vacuolated. The cytoplasms of these cells usually stained darkly and evenly

except around the nuclei where large vacuoles are lightly staining granular cytoplasm were observed. This vacuolization is much more noticeable than that described as surrounding the nuclei in a normal midgut.

The mid cells, possibly because of the stretched epithelial layer, seem to have been reduced in size and therefore, were difficult to distinguish. The striated border is disrupted by the elongate cells and could be seen only occasionally in the hollow between the cell tips. It usually appeared as a granular mass with knows apparent striations.

The epithelial cell contained a number of large vacuole especially around the nuclei and the cytoplasm seemed to thin out, stain light and appear granular. The proliferation of vacuolated elements by the epithelial cell appeared greater in midgut containing the gas bubble. The nuclei of practically all cells stained darkly and uniformly.

4.DISCUSSION

The primary functions of the foregut first appear to be mechanical, but there is a doubt that the organ in insects has secondarily come to be also a physiological adjunct to the stomach by increasing the space available for digestive purposes. Since the food is stored in the crop is subject to the actions both of the salivary liquid swallowed and mixed with the food during ingestion and gastric juices that flow forward into the crop from the ventriculous The circular muscles, as the stratified layers, covered the large sclerotized rows of spines to ensure the grinding and the contracting action required from the muscles at this stage of digestion. The internal longitudinal muscles area are composed relatively of a very thin layer of muscles which are difficult to observe in this area that is largely dominated by a mass of very active circular muscles. Also there are no observed external longitudinal muscles in this region-the muscles may be embedded underneath or within the mass of circular muscles. The structural organization of the alimentary canal of *M. pustulata* was more or less similar to that of *Dendroctonus paralleocollis, D. rhizophars* and *D. valens* (Elba Diaz *et al.*,1998) and other coleopteran insects (Zungia *et al.*,1994) However, the species studied showed distinctive characteristics. The arrangement of gastric caeca of *M. pustulata* also similar that observed *D. valens* and D. adjunctions (Zuniga *et al.,* 1994) and supports the proposal to use this characteristic as an additional taxonomic character (Thomas, 1967).

In the present study it has been proved clearly that histologically the wall of pharynx and oesophagus is made up of an epithelium of uninucleate cuboidal cells lined by chitinous intima and circular muscle fibres. This appears to be similar to that of other coleopteran insects such as *D. parallelocollis, D. rhizophagus, D. valens ,D. adjunctus* (Zunuga, *et al.,* 1994) and that the same characteristic appeared in other orders such *D. cingulatus* (Muralitharan 1983) and *Chrysochoris purpureus* (Baskaran, 1970). But the presence of crop and proventriculus is not to be correlated in other order such as hemipteran insects, and it is to be correlated with biting and chewing habit of this insect.

Furthermore, *M. pustulata* have the inner longitudinal muscle layer that has been observed in *D. adjunctus* (Zuniga *et al.,* 1994) and other coleopteran such as *T. lineatum, G. retusus, G. sulcatus Leconte* (Schneider and Rudinsky, 1969a), but did not have the same other coleopteran such as *D. parallelocollis* and *D. valens*.

The anatomy and histology of the crop were similar in *M. pustulata* as in *D. parallelocollis, D.rhizophagus, D. valens* and *D. adjunctus*. But the histological features were different in group of beetles because the division in the current study of the crop was based on the presence of non sclerotized grooves. Unlike the findings reported for other Scolytid coleopteran species, gas bubbles in the crop or proventriculus, associated with the various responses of the insects to environment during flight, were not found in the *M. pustulata* studied.

The midgut region in *M.pustulata* is proved with simple column epithelium showing tendency of folding towards posterior site. The epithelial cells also characterized the midgut into two parts. Simple columnar epithelium is reported throughout the length of the midgut *Onthophagus catta* and *Aphodius maestus* by Verma (1969). The histological differences were noticeable in the epithelium, especially in relation to the cell size, secretary vesicles, the cellular nidi. The reports for *T. lineatum, G.retusus, S. multistriatu* and *D. adjunctus* coincide with these results, and show that these region is highly variable (Zuniga *et al.,* 1994).

The histological characteristics of the midgut valve or stomadeal valve of *M. pustulata* were similar and organization and arrangements of its structures coincided with those reported for other coleopteran insects (Zunuga *et al.,* 1994). The structural and secretary richness found in the midgut confirms the importance of this region in the digestive process (Terra,1990).

The anatomical and histological structure of the hind gut of *M. pustulata* was similar and coincided with that reported for other coleopteran insects such as *D.adjunctus* and *D. parallelocollis* (Zunuga *et al.,* 1994). However, in the rectum there were differences in the epithelium, in the species studied forms wide and short folds whose number and arrangement are similar to the rectal pads described for other coleopteran insects.

Sharma (1996) while studying the effect of parathion on the alimentary canal of male *Poecilocerus pictus* F. reported that foregut was more affected than the hind gut. Lashman Lal *et al.* (1970) reported that as a result of feeding of endosulfan, diazinaon and dichlorves, to the fourth instar larvae of tobacco caterpillars intima was not affected, whereas disintegration, degeneration, vacuolazition, disappearance of the cells of a poor degree and shedding of cytoplasm was observed in the ooesophagus and crop. The present findings agree with the findings of Sharma (1966) and Lakshman Lal (1970) except the intima and crop, which was also damaged in the present observation.

The midgut unlike foregut is considered most susceptible to almost all stomach poisons. The peritrophic although resistant to most insecticide has been reported susceptible to parathion

by Salkeld (1950) in the honey bee but Woke (1940) reported no effects of lead arsenate and Paris green on the peritrophic membrane of the army worm larvae. The present investigation in *M. pustulata* shows the peritrophic membrane heavily damaged by Azadirachtin treatment.

The epithelium of midgut of several insect has been found to be affected drastically by different insecticides as reported by Srivastava (1962), Rizvi and Khan(1973), Shukla *et al.*, (1977), Sabesan and Ramalingam (1978) and Zutshi and Saxana (1989). In *M. pustulata* according to observation, the administration of Azadirachtin exhibits prominent effects at lower concentration. Although, vacuolization, hypersecretion, pycnotic condition of epithelial cells and the detachment of muscle layers are prominent. Shukla *et al.*, (1977) and Zutshi and Saxena (1989) have reported the effect of pyrethrum on the midgut of *Aulacophora foveicollism* and *G. sigillatus*, which included the degeneration of the regenerative crypts, affecting both the circular and longitudinal muscle fibre. Muckherji and Hardas (1954) reported that of 3rd instar hopper of *Schistocera gregaria* F. was degenerated of the muscle layer as a result of parathion poisoning. Similar observations were reported by Lakshman Lal *et al.*, (1970) in case of caterpillar of *Spodoptera litura* F. when treated with diazinon. Soliman and Soliman (1958) reported that parathion, when fed to 5th instar larvae of *Prodenla litura* F. completely destroyed the midgut epithelium. But the present investigation failed to fully support their findings as mid gut was neither degenerate nor completely destroyed.

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