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EFFECT OF POLYTRIN-C AND NUVACRON ON THE ENTERIC EPITHELIUM OF *DYSDERCUS CINGULATUS* (FAB.) (HEMIPTERA: PYRRHOCORIDAE)

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Abstract: The adults of *Dysdercus cingulatus* were treated with 1.7, 0.8, and 0.4 ppm concentrations of Polytrin C in acetone whereas, 2.2, 1.1, and 0.6 ppm doses of Nuvacron were used. All the doses were given for 12 & 24 hours.

LD_{50} for Polytrin C and Nuvacron was calculated to be 3.31 ppm and 4.37 ppm, respectively afrer 24 hours of the treatment. The effect of both Polytrin C and Nuvacron was found almost the same. Midgut seemed to be more effected as compared to the the fore- and hind-guts. Both insecticides disrupt the epithelial lining of the gut. Cells became elongated and narrow after treatment with smaller concentrations. Vacuolization also took place. Higher doses of insecticides had more acute effect. The cells lost all their contents and became flattened. They had almost no cytoplasm and the nuclei seemed to congregate.

Peritrophic membrane which was present in the midgut of control insects also broke-up and became absent over most of the surface. Cuticular intima of the foregut and hindgut became thinner and damaged. It broke-up in the foregut but in the hindgut it only became thinner.

Key words: *Dysdercus cingulatus*, Polytrin C, Nuvacron, insect entric epithelium, insecticides.

INTRODUCTION

The cotton crop is prone to attack by a large number of major insect pests resulting in a considerable reduction in yield per acre. Various workers have given different estimates for the losses caused by insect pests to cotton crop. Hague (1972) estimated that different pests, on an average, cause 5-10% damage to cotton crops every year. Ghouri (1973) was of the view that cotton suffered the loss of about 16-20% due to this cause, while Chaudhry (1976) assessed this loss up to 32%. Ahmad (1983) and Yasmeen *et al.* (1985) have also discussed the increased damage to cotton crop by pests.

Dysderous cingulatus is widely distributed in Pakistan. Apart from cotton it also feeds on ladyfinger, maize, pearl-millet, wheat, clovers *etc*. The insects suck cell sap from leaves and green bolls of cotton. Heavily attacked boll open badly and the lint is of poor quality. The cotton also gets stained by their excreta. The seeds thus produced have low rate of germination. Moreover, the lint is stained further with their excreta or body juices as they get crushed in the ginning factories. The staining to the lint by the growth of certain bacteria inside the bolls is also believed to be initiated by the bugs. The chemical control is the most popular method, and different agents ranging from chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids have been introduced one after the other. Partial control is the minimum which can be achieved.

The development of resistance in insect population against various insecticides is the main reason for the failure and has forced the workers to use different biologically active chemicals against them.

The effect of the insecticides more or less depends upon the route of administration, it may be oral, tropical or intravenous (Awad and Kandil, 1980; Bariola *et al.*, 1984; Fahmy *et al.*, 1985; Powell *et al.*, 1980).

As the intestinal cell lining palys an important role in digestion, absorption and detoxification (Richards and Davies, 1977; Rastogi *et al.*, 1987) when any insecticide is given orally or topically, it is one of the tissues which is affected most. For this reason during the present study the entire epithelium of this adult bug was studied before and after the use of Polytrin C and Nuvacron.

MATERIALS AND METHODS

The red cotton bugs, *Dysderous cingulatus* were collected from cotton fields at Bhai Phero, and from the vicinity of New Campus, Punjab University, Lahore. They were brought to the laborataory where they were sorted out according to their age. The different nymphal instars and adults were kept in separate jam jars and covered with muslin cloth. Small green leaves of different plants, especially those of cotton and shoe flower plants were given them as food. In this way a constant supply of the adult bugs was ensured. These insects were maintained in the laboratory at a temperature ranging 25 - 30^{O}C and 70$\pm$5% relative humidity.

Insecticide treatment

The adult bugs were treated with selected concentrations of Polytrin C (1.7, 0.8, and 0.4ppm) and Nuvacron (2.2, 1.1, and 0.6ppm) for 12 and 24 hours. Three grams of green leaves of cotton were soaked with 3.7 ml of each concentration of insecticides in petridishes. They were transferred to jars alongwith 10 adult bugs. Thus the insects fed on the treated food also came in body contact with the contaminated leaves. Besides the treated, the untreated control experiment was also carried on. For control, 10 adult bugs were fed on the untreated food.

Histological Studies

The adult bugs were dissected in 0.75% saline solution. Their alimentary canals were removed and put in watch glasses in the saline solution. The connective tissue present on the surface of the gut was scrapped off by means of a soft camel-hair brush. Different parts of the gut (fore-gut, mid-gut and hind-gut) were separated and fixed in Bouin's alcoholic fixative, embedded in paraffin wax and sections were cut serially with rotary microtome at 5μ. They were stained with Mallory's Triple Stain and hematoxilin which was counter stained with eosin.

RESULTS

Alimentary canal of control insects

The alimentary canal of *D. cingulatus* is a very long convolvulated tube extending

from the mouth to the anus. It is more than double the body length and typically divided into a fore-gut, a mid-gut and hind-gut. The Malpigian tubules are present between the junction of the mid- and hind gut (Figs. 1,a-c).

Fore-gut is the short portion of the alimentary canal. Its internal walls are thrown into many folds which are variable in depth. Internally the foregut is lined by a thin cuticular layer outside which is the epithelial layer. Its cells are normally tightly packed and either cuboidal or columnar with large nuclei. The folded parts are sometimes multilayered with tightly packed cells of varying dimensions. Their length varies from 16-20μm, while their width range is from 5-7μm. The nuclei which are generally centrally placed or slightly towards the base are rounded and 4-5μm in diameter. The cells have granular cytoplasm which sometimes may be slightly vacoulated. The chromatin is scattered randomly. They rest on a very thin basement membrane externally. Its thickness is never more than 0.5μm. The epithelial layer is surrounded by an inner layer of circular and an outer layer of longitudinal muscle fibres. They are well developed over the pharyngeal region. but over the oesophagus and the crop the longitudinal muscle fibres are poorly developed or even absent. The outermost peritoneal covering is very thin and almost non-existent. It has some tracheal supply. Haemocytes could be seen adhering to it at places.

Midgut is divided into two parts. The anterior part is in the form of a long tube while the posterior part is short.

The anterior midgut is a narrow and clearly demarcated from the foregut. It is lined internally by a very thin peritrophic membrane which is from 0.5 - 1.0 μm thick; outside this lies the epithelial layer which is of columnar cells. They are narrow and tall with a length range of 12-14μm and a breadth range of 4-5μm. They have granular cytoplasm. Their nuclei are small, only 3-4μm in diameter. They lie toward the basal part of the cells. The cytoplasm was seen to be vacoulated occasionally. Secretary granules could also be seen flowing into the lumen. The epithelial layer rests on a thin basement membrane which is around 0.5μm thin. The muscular layer is very thin, composed of an inner layer of longitudinal and an outer layer of circular muscle fibres. The wall of this part is also thrown into folds.

The posterior midgut region of the midgut has the same layers as the anteior portion, except that the cells show greater evidence of secretory activity. They are less tall, around, around 9-10μm in length, with well defined generally rounded or ovoidal nuclei. They are from 3 - 4μm in diameter, with conspicous uniformly scattered chromatin material. Big vacuoles are sometimes present in the cell.

A second type of cells, the regenerative cells are also found lying beneath these cells singly or in groups of two's or three's. At places a well defined space filled with granules is present between the epithelial and muscular layers. The muscular layers form many small diverticula with the continuation of this space and material inside them. This region of the midgut is also thrown into folds which are larger here as this region is aslo larger in diameter as compared to the anterior part. Enteric caeca were aslo present in this region.

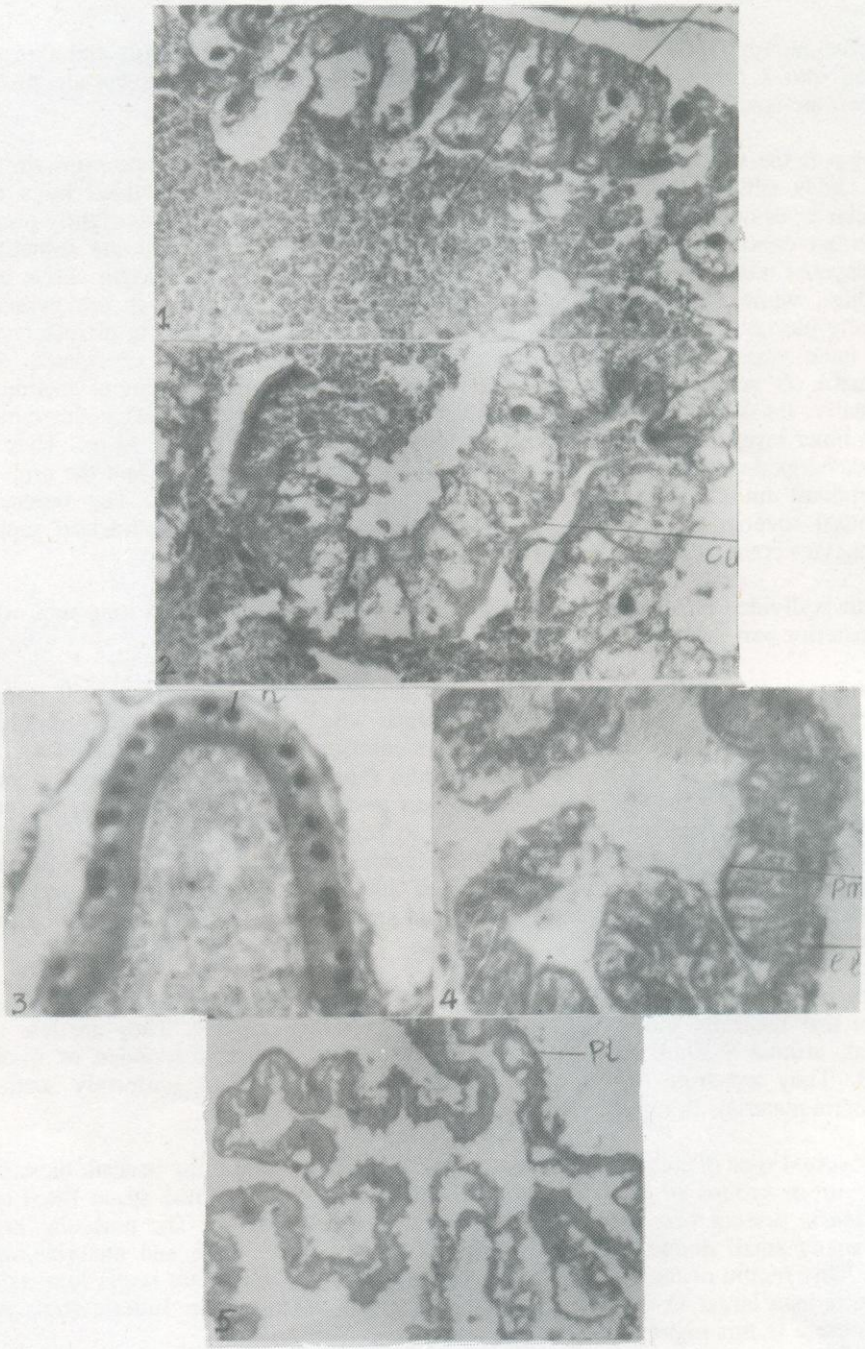

Fig. 1. T.S. of the various parts of the alimentary canal of red cotton bug. a, foregut showing large epithelial cells, 400x; b, foregut showing folding of epithelial lining, 400x; c, anterior midgut, 200x; d, posterior midgut showing darkly stained nuclei, 200x; e, hindgut, 200x

Hindgut is a short tube with a thin muscular layer. The epithelial layer has very narrow tall cells with small nuclei lying generally towards the base. Multinucleate cells are also common. These cells have many tightly packed vacuoles towards the lumen. The cuticular intima is very thick at places. Muscular layer is very poorly developed but peritoneal layer is well defined.

Alimentary canal of treatede insects

After treating the bugs with different concentrations of Polytrin C and Nuvacron for 12 and 24 hours, their effects on gut were studied (Figs. 2, a-e).

The effect of both the insecticides wa almost similar on the gut cells. Prolongation of the treatment intensified the effect. Generally the cells of the epithelial lining of the foregut became elongated and vacuolization took place. Twelve hours after treatment the epithelial cells which were squamous or columnar became tall and narrow, with an average height of 20μ and 3.5μ width. The nuclei either became elongated and narrow (6μ x 2μ) or decreased in size up to a diameter range from 1.5μ to 2.0μ. Vacuolization took place and clumping caused the presence of bigger granules in the cytoplasm. Partitioning of the cytoplasm also took place, so that the epithelial lining seemed multilayered at some points. Muscular layers were found to be the least affected.

After 24 hours of treatment, breakage of the too much elongated cells had taken place, and each cell of the inner layer had vacuoles towards the inner ends. Much cytoplasm had oozed out of the cells forming a regular layer, thus completely lining the lumen. The cuticular intima was consequetly all disrupted. The folds had become elongated and fewer.

The effect of these insecticidal doses on the midgut was the same. Here the epithelial lining was the most affected and the cell secretions had flown freely into the lumen. The muscular layer had become non-existent and no peritrophic membrane could be detected. In the hindgut also disruption of the cellular layer was quite conspicous but the cuticular layer was hardly disrupted.

Effect of higher doses

Higher concentration of the insecticide after 12 hours of the treatment had a similar effect as described for the lower dosages but of greater intensity. Many cells had become binucleate and general disruption and histolysis of the epithelium had taken place. At places the cellular layer had almost disappeared and only vacuoles could be seen. After 24 hours of the treatment the epithelial cells generally had become flattened all over the alimentary canal and cell boundaries had mostly diappeared. Cuticular intima of the foregut was found very much disrupted or absent and in the hindgut also it had become tinner. Pritrophic membrane could not be detected at all.

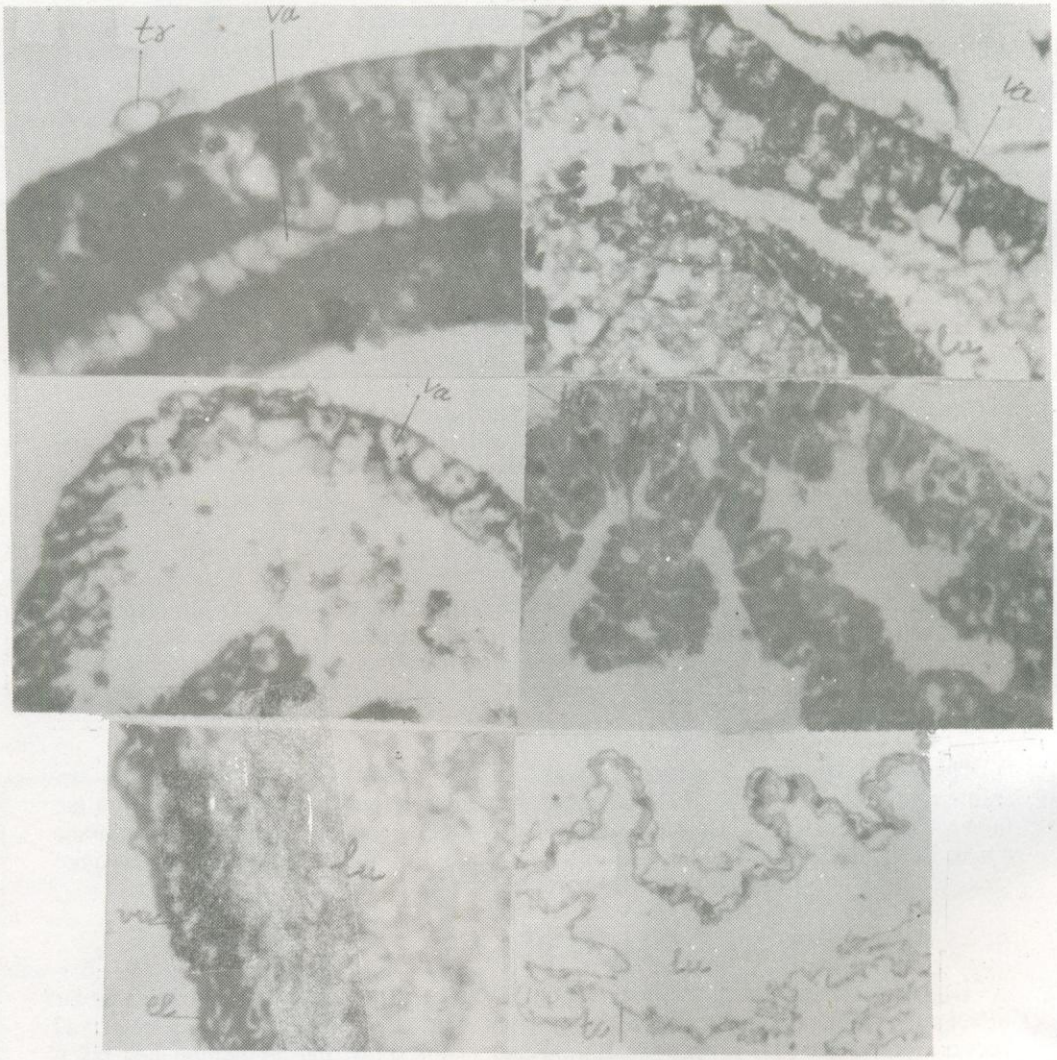

Fig. 2. T.S. of various parts of the alimentary canal of insecticide treated red cotton bug: a, foregut, treated with Polytrin C (0.8ppm) for 24 hours, 400x; b, the anterior midgut treated with Polytrin C (0.8ppm) for 24 hours, 400x; c, the posterior midgut treated with Polytrin C (1.7ppm) for 12 hours, 200x; d, the foregut treated with Nuvacron (1.1 ppm) for 12 hours showing epithelial layer, 400x; e, the posterior midgut treated with Nuvacron (0.6 ppm) for 24 hours, 400x; f, the hindgut treated with Nuvacron (2.2 ppm) for 24 hours showing cellular layer, 200x.

Abbreviations used: tc, thin cellular layer; lu, lumen; cu, cuticular layer; n, nucleus; el, epithelial layer; pm, peritrophic membrane; pl, peritoeal layer; va, vacuoles; tr, trachea; bn, binucleate epithelial cells.

DISCUSSION

The alimentary canal of the adult red cotton bug is very long and tubular as is generally the case with fluid feeders (Chapman *et al.*, 1985; Richard and Davies, 1977). In this bug, apart from the pharynx, all the other parts of the gut have a very weak or no muscular layer at all. The midgut which is the main site of digestive activity is divided into an anterior and a posterior part. The division of the midgut into different portions is also common in fluid feeders especially those feeding on sap *i.e.*, petatomorphia has 4 recognizable divisions, or ventriculi while some others have three (Chapman, 1971; 1985). These divisions help to get rid of the excessive water content of the food as it flows along their length. Development of other devices like filter chamber in some insects like Cercopoidea is for the same purpose, otherwise the blood can becomes too diluted and thin. The ingested water enters the blood via the gut epithelium and other histological layers.

A fairly well defined peritrophic membrane was also found lining the midgut epithelium. It is said to be absent from most insects feeding on a liquid diet. However, it is present in *Cicadella*, mosquitoes and *Glossina* (Gouraton and Maillet, 1985; Moloo et al., 1970; Freeman, 1973) and accordig to Waterhouse (1953) may have been overlooked in other fluid feeders. It may have some other function apart from protection from food and act as a selective membrane and be secreted in response to a stimulus. This was first proposed by Stohler (1957) but later on many workers investigated it in various insects like *Simulium*, (Fallis, 1964; Lewis, 1950), Culicoides (Megahad, 1956), *Phlebotomus* (Gemetohu, 1974). In mosquito even a small blood meal can induce its secretion and a second meal can also stimulate the production of a second membrane which surrounds the first membrane (Waterhouse, 1953; Freyvogel and Jaquet (1965). Richard and Richard (1977) have given many other examples of this type of behaviour by insects. It seems that in the red cotton bug also the peritrophic membrane is secreted in response to feeding.

Alimentary canal plays an important role in the detoxification of various insecticides used in the field. The insecticides do not enter the insect body by cuticular penetration only, they are also ingested with the food and reach the haemolymph and target and non- target organs after passage through the gut wall. Transport through the gut wall is a passive deffusion process, because neither the presence of inhibitors of carbohydrate metabolism or couplers of oxidative phosphorylation, nor even the absence of oxygen has any effect. Its rate may be different in the different insects and also depending upon the type of insecticide (Shah and Gutherie, 1970; 1971).

In the red cotton bug Polytrin C and Nuvacron had almost the same effect on the gut. All parts of the alimentary canal were found affected. The maximum disruption took place in the epithelium lining of all gut divisions, especially those of the midgut. Higher doses and prologed treatment intensified the effect. Ultimaltely the enteric epithelial cells became flattened and vacuolated, with almost no cytoplasm *i.e.* histolysed to an extensive degree. The midgut epithelium is the site of secretion and absorptive activity. So this was observed to be the most vulnerable part. These insecticides proved fairly successful against this bug.

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