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Histopathological studies of some insecticides on the alimentary canal of Khapra beetle *Trogoderma granarium* (Evert)

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Abstract- The present investigation describes the histopathological changes of different sections of alimentary canal of *Trogoderma granarium* (Evert) due to effect of experimentally ascertained LD_{50} doses of Gamma-BHC, Malathion and Fenitrothion. The uses of insecticides for effective and prompt control of the harmful population of insects are widely prevalent now a day. Their extensive use in pest management is mainly due to their simplicity, promptness, effectiveness and flexibility in character. The histopathological study of alimentary canal of different insects due to the effect of the insecticides has been studied by various workers such as Pilat (1935)[1], Woke (1940)[2], Eckert (1948)[3], Lal *et al.* (1970)[4], Misra and Mukherji (1972)[5], Misra *et al.* (1972)[6] & Mukherji and Misra (1972)[7]. In the present investigation, histopathological changes of alimentary canal of *Trogoderma granarium* (Evert) with Gamma-BHC, Malathion and Fenitrothion have been studied.

Key words: Khapra beetle, Pest, Alimentary canal, LD_{50} doses, Insecticides, Maize.

INTRODUCTION

Insect is the oldest animal in this world, found almost everywhere, often in huge numbers. As the Khapra beetle is one of the most feared stored product pests. Population of this pest can build rapidly in the short time under hot and dry conditions. Grain damage depending on existing conditions often reaches upto 70% damage has been reported. It prefers grain and cereal product particularly maize (*Zea mays*). Generally insects may annoy for human beings but on the other hand, they are very helpful in various ways for instance in removing dead animals, producing honey, wax, lac, silk etc. It is roughly estimated that the annual loss due to various pests affecting our crops is about one thousand five hundred crores rupees.[8] However, insects are of great significance to mankind because they affect the human economy to a large extend. Therefore, the annual loss to the stored grains is estimated to be around upto 20% due to inadequacy of storage. The major insect pest of stored grain is *Trogoderma granarium* (Evert). Its tissues are selected for the investigation of the foregut, midgut and hindgut. The present investigation deals with the histopathological changes induced by the selected insecticides on the different parts of the alimentary canal of the maize pest *Trogoderma granarium* (Evert).

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MATERIAL & METHODS

Laboratory culture of the insects was raised on maize (*Zea mays*) at 30±2°C temperature and 70 ±5 % R.H. only newly immerged adult insects were used in the experiment.

The insecticides used in the investigation were the technical grades of Gamma-BHC, Malathion and Fenitrothion. Experimentally ascertained LD_{50} doses of Gamma-BHC, Malathion and Fenitrothion were administrated into the alimentary system of each insect by means of micro drop applicator. The moribund stage of the test insects reached after about 10 hours of the treatment. These insects were fixed in the alcoholic Bouin's fixative after cutting the legs and elytra with the help of a very sharp blade. Paraffin blocks were prepared followed the standard procedure of dehydration and paraffin embedding at 58°C. During final stage of dehydration and clearing, instead of absolute alcohol and xylene, 96% alcohol and amyl acetate were used. Sections were cut at 8µ. The usual double stain technique of haematoxylin and eosin was used for staining the sections. Control slides of untreated insects were also prepared by followed the same procedure.

OBSERVATIONS

The following histopathological changes were observed in the different sections of alimentary canal of adult *Trogoderma granium* when treated with LD_{50} value of gamma-BHC, Malathion and Fenitrothion.

(1) Effects on the Foregut:

No histopathological changes were observed in the section of alimentary canal due to the effects of insecticides used in the experiment.

(2) Effects on the Midgut:

(a) Gamma-BHC Poisoning:

Little changes were observed in the epithelial layer of the midgut. The epithelial cells showed signs of shrinkages of cellular cytoplasm. Due to this, a number of small vacuoles appeared in the cells. The boundaries, of the cells were not distinct due to shrinkages. At these places the shrinkage of cytoplasm resulted in the detachment of basement membrane from the epithelial cells. Nuclei of the epithelial cells were also displaced, hence not distinct clearly. No significant changes were detected in the muscular layer (Fig. no. -3).

(b) Malathion Poisoning :

(i) Upper part of the midgut:

The epithelial layer was greatly affected due to Malathion poisoning. These cells showed their complete detachment from the basement membrane. At many points, due to longitudinal splits epithelial layer was broken into fragment were found to be thrown into lumen. In the broken fragment of epithelial layer, the nuclei were also found to be displaced. Muscle layers also showed signs of shrinkages (Fig. No.-4).

(ii) Lower part of the midgut:

The histopathological changes due to Malathion poisoning was not found to be the same throughout the length of the midgut. In the lower part of the midgut, different changes were observed. At a number of places, the epithelial layer got greatly folded due to shrinkage. As a result of this, the lumen was found to be greatly reduced. Large number of small vacuoles also appeared in the epithelial layer. This resulted due to the displacement of the nuclei. The basement membrane and muscle layers were greatly fused with the epithelial layer due to the shrinkage. (Fig. No. -5).

(c) Fenitrothion Poisoning:

Fenitrothion poisoning showed marked histo pathological changes throughout the length of the midgut. The epithelial layer was found to be completely detached from the basement membrane. Due to longitudinal splits, this layer was found to be broken at many points. Epithelial cells were also found extended. Due to this, nuclei were displaced and moved more towards the basement membrane. The end of the epithelial cells towards the lumen became spindle-shaped. No significant changes were observed in the muscle layers (Fig. No. -6) .

(3) Effects on the hindgut:

This section of alimentary canal was found to be affected by Gamma-BHC poisoning. The cuboidal epithelial cells got folded and greatly shrank, as a result of which lumen were reduced. No significant changes were seen in the muscle layers (Fig. no. -7).

RESULTS AND DISCUSSION

Foregut of the alimentary canal was not affected by any of the three insecticides. The midgut region showed marked histopathological changes due to effect of all the three insecticides gamma-BHC only. Effects were of varying degree of vacuolations, detachments, splitting and shrinkages in the epithelial layer of midgut whereas, only shrinkages folding and thereby reduction in the size of lumen were found in the hindgut.

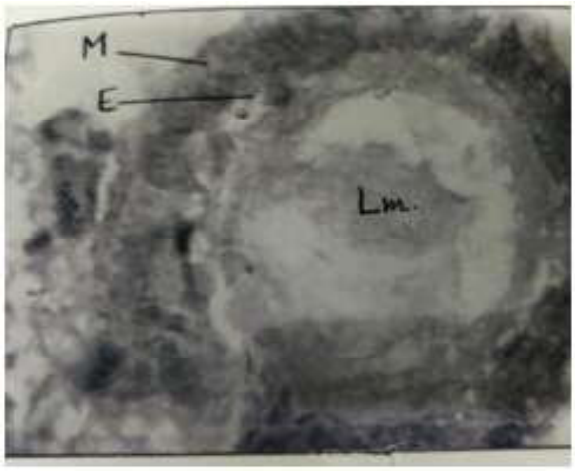

Fig . no. 1-T.S. of Midgut showing normal structure.

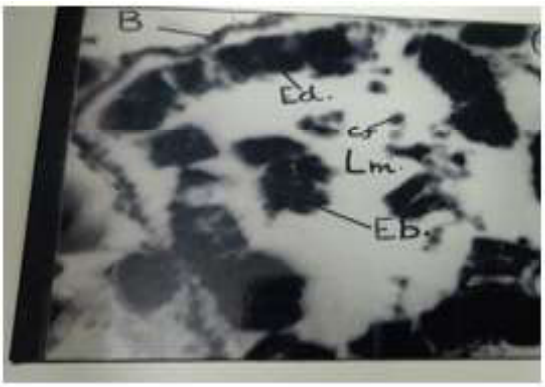

Fig. no. 4- .T.S. of upper part of the midgut treated with Malathion showing detached the broken epithelium (X 400).

Fig. no.2-T.S. of hindgut showing normal structure.

Fig. no. 5- T.S. of lower part of the midgut treated with Malathion showing greatly folded and vacuolated epithelium having displaced nuclei and reduced lumen (X 150).

Fig. no. 3- T.S. of midgut treated with gamma-BHC showing signs of shrinkage and detachment of epithelial layer (X 150).

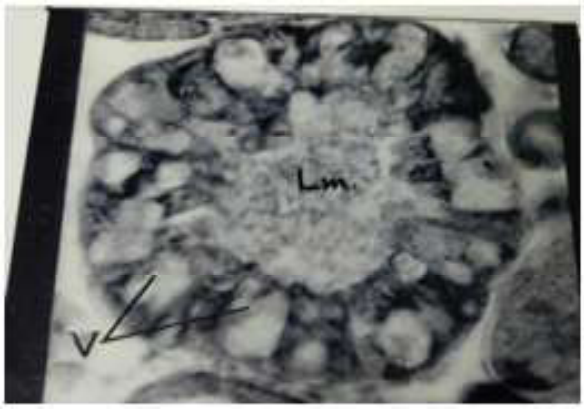

Fig. no. 6 – T.S. of midgut treated with Fenitrothion to show completely detached epithelium longitudinal splits and elongation of epithelial cells having displaced nuclei (X 400).

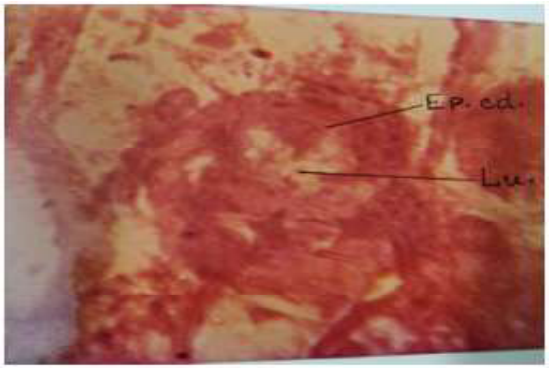

Fig. no. 7 – T.S. of hindgut treated with gamma-BHC showing greatly shrunk and folded epithelium and very reduced lumen (X150).

Similar findings on the foregut were found by Brown (1951)[9] and Steinhans (1967)[10]. The cause of no marked histopathological change in this region may be due to the presence of intima and retaintion of the insecticides for a very little time in this region of alimentary canal. But on the other hand, marked histopathological change in this foregut, due to the effect of various insecticides have been observed by Sharma (1966,1968)[11,12] in *Poecilocercus pictus* and *Dysdercus koenigie*, Lal *et al.* (1970)[4] in *Spodoptera litura*. Misra and Mukerji (1972)[6] in *Drosophila melanogaster*.

Since the midgut epithelium is physiologically the most active site of the alimentary canal, most of the digestion of the food and absorption of the digested food take place in this region (Metcalf and March,1953; O´ Brien,1957)[13,14]. Muscle layers have shown the sign of shrinkages and their fusion with the basement membrane in the lower part of the midgut. Steinhans (1967)[10] has suggested that the histopathological changes in the midgut are variable. Minor to serious damages of similar nature in the cells of midgut of *Vanessa uticae*, *Prothetria dispar* and *Pieris brassicae* have been also reported by Pilat (1935)[1]. Woke (1940)[2] has observed disintegration of entire epithelial cells of the midgut in *Prodenia eridania* due to arsenic poisoning. Soliman and Soliman (1958)[15] have also described the changes in the midgut in the larvae of *Prodenia litura* due to parathion poisoning. Mukherji and Hardas (1954)[16] and Soliman and Shehata (1963)[17] have studied the effect of parathion and other chlorinated hydrocarbons on second nymphal instar of *Schistocerca gregaria* and larvae of *Musca domestica* respectively. They have reported the complete disintegration of midgut epithelium in both the cases. Hassanein and Khalil (1969)[18] have reported elongation of the midgut of *Coccinella undecipunnctata* due to metasystox poisoning Toppozada *et al.* (1968)[19] have also reported the histopathological changes in the epithelial cells of the *Spodoptera littoralis.* Datta and Das (1971)[20] and Misra and Mukerji (1972)[6] have reported similar findings in the midgut of *Periplaneta americana* and *Drosophila melanogaster* respectively. Sah and Srivastava (1989)[21] have reported the histopathological changes of different sections of alimentary canal of pulse beetle due to the effect of experimentally ascertained LD50 doses of different insecticides. Although it is difficult to point out the exact cause for histopathological changes, but it can be concluded that with all probability, all the marked changes, such as, detachment of epithelial layers from the basement membrane, vacuolation and longitudinal splitting of the same, displacement of nuclei of the epithelial cells from their original position are due to shrinkage of the cells caused by the loss of water.

The changes in the hindgut have been observed only due to gamma-BHC poisoning. The changes may not be considered to be of much significance, when compared with that of the midgut. Because it causes only reduction in the lumen due to the folding and shrinkages of cuboidal epithelial cells. The vacuolation in the epithelial layer and its detachment from the basement membrane and the displacement of nuclei from their position are not seen. This may be due to the thickness of intimae in this region. The other two insecticides used in the experiment can be completely utilized in the midgut causing severe damage there, hence, they do not reach the hindgut in significant concentration to cause any damage to this part.

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