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Research Article

EFFECT OF CHROMAFENOZIDE ON THE ULTRAMORHOLOGY OF LARVAL MIDGUT OF *SPODOPTERA MAURITIA* BOISD. (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

In the present study, the effect of the sub-lethal dose of chromafenozide (non-steroidal ecdysone agonist) was studied on the morphology of the midgut, using scanning electron microscopy. The sub-lethal dose of the compound caused severe damage in the muscular layer of midgut. In the outer surface of midgut, longitudinal muscle showed only few notable changes than the circular one, with respect to the time of application. After treatment, the inner surface of midgut exhibited significant changes in the cytoplasmic protrusions. The ultra-morphological features of midgut, with the morphometric measurements exhibited significant reduction in the width of longitudinal muscle bundles. Scanning Electron Microscopic images revealed the intensity in the level of degradation and damage caused to the mid gut surface areas, when treated with chromafenozide.

INTRODUCTION

Insect growth regulators based on hormones have important value as pesticides of the future, and also acts as outstanding chemical probes to explain the role of hormones in the basic physiological processes of insects. Insect moulting hormone (20-hydroxyecdysteroids) is one of the major hormones in the regulation of growth and development of insects. The first insecticide with ecdysone agonist activity was developed in 1991, and to date, four highly potent compounds were commercialized. The development of bisacylhydrazine have stimulated intensive research in the field of insect endocrinology, emphasizing on biological activity of ecdysteroids and non- steroidal ecdysone agonists in whole insects, tissues and cells (Oberlander *et al*., 1995; Dhadialla *et al*., 1998). Several studies were shown that both tebufenozide and methoxyfenozide are selective in action on lepidopteran insects whereas halofenozide is found to be more effective on coleopteran (Nakagawa, 2005). Though, several studies have been conducted on the effects of the various ecdysone agonists on physiological and reproductive aspects, but a detailed study has not envisaged with the newly developed ecdysone mimic, chromafenozide.

The digestive system of insects is mainly concerned as a natural and chemical defense barrier against many foreign invasions of toxic compounds. Morphological studies act as an important tool, for understanding the form of action of any toxic compounds. Many of the deleterious and physiological effects are measured by analysis of the growth; reductions and abnormalities of the tissue concerned (Mordue and Nisbet 2000). Therefore, in this context, the present investigation was aimed to provide requisite back ground information on the external morphology of larval midgut of *Spodopteramauritia* as affected by the sublethal dose of chromafenozide.

MATERIALS AND METHODS

Chemicals & Treatments

The nonsteroidal ecdysone agonist chromafenozide, purchased from Sigma Aldrich were used for the study. The compound was dissolved in acetone and diluted to obtain the required concentrations.

The last instar larvae of *Spodopteramauritia* were treated topically with different doses of ecdysone agonist, using a Hamilton microsyringe. In topical treatments, the acetone solution containing the non-steroidal ecdysone agonist chromafenozide was applied topically along the dorsal midline of the abdomen of the larvae. An equivalent volume of acetone was applied to control larvae in a similar manner.

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Dissection and fixation of tissues

Sixth instar larvae were mildly anaesthetized in specimen tubes, taken out from the treated and control colony after 24 and 48 hours. The larvae were taken out and pinned dorsal side up in a wax-lined petridish, immersed in insect Ringer solution (Ephrussi and Beadle, 1936). A longitudinal cut was made on dorsal surface; using sharp surgical scissors and the midgut were removed.

Scanning Electron Microscopy

Tissue Preparation

The midgut of treated and control larvae were fixed in 2.5% glutaraldehyde, containing 1.8% sucrose in PB at 4^0C overnight. After 12 hours, tissues were washed in a graded ethanol series of 50%, 70%, 90% and 100%, then washed in 100% acetone.

SEM imaging

The tissue was individually mounted on aluminium stubs with double-sided sticky tapes. Dehydration process was followed by air drying in a drying cabinet conditioned to 25 ± 1^0C and 10 ± 1% RH. The tissues were then sputter coated with gold in a Polaronion sputter coating unit. The specimen were examined in Hitachi SU6600 Variable Pressure Field Emission Scanning Electron Microscope (FESEM), set at 10kV (Electron gun: Tungsten Schottky emission electron source, Resolution: 1.2 nm/ 30 kV, 3.0 mm/ 1 kV, Probe current: 1pA~200nA, Specimen chamber pressure: 10-4 Pa (high vacuum) and 15mm working distance. Both outer and inner surfaces of the midgut were observed and photographed at different resolutions.

Morphometric Measurements

Morphometric measurements were performed using Axiovision software package. The obtained data were analyzed using student's t-test in SPSS 16.0 software. The outcome of the test expressed as Mean ± Standard Deviation (standard error of mean).

RESULTS

Effectof Chromafenozide on the outer surface of the midgut

The sub-lethal dose (0.1µg/5µl) of chromafenozide was selected for examining the ultramorphological changes in the midgut structure of the treated sixth instar larvae. Midgut of larvae dissected after 24 and 48 hours of post treatment with Chromafenozide and control samples were examined for scanning microscopic studies.

Midgut of day 1 (24 hour) and day 2 (48 hour) sixth instar normal larvae (control) were examined for the ultrastructural study. Generally, the muscular layer of the midgut of lepidopteran insects is less developed than the foregut. It is constituted by internal circular layer and an external longitudinal one. Examination of longitudinal muscle layer of sixth instar normal (control) larvae of 24 and 48 hours (Figs: 1, 2 and Figs: 5, 6), are thick and continuous. Circular muscle also has intact structure.

Scanning microscopic examination of sixth instar larvae, treated with chromafenozide (0.1µg) after 24 hours, displayed (Figs- 3, 4) no distinct change in both muscular layers. In circular muscles, the edges of the bundle, exhibited some degree of disintegration, which resulted in appearance of a separation in between the bundles. Longitudinal muscles have small damages as small pores (Figs- 3, 4). These damages are more evident in chromafenozide treated larvae after 48 hours of treatment (Figs: 7, 8) on the longitudinal muscle layer small pores are present along the layer. The separation in between the circular muscle layer is more evident and it turned to a clear depression.

Effects of chromafenozide in the inner surface of the midgut

Generally the inner surface of the midgut reveals the cytoplasmic protrusions and the minute brush border of the columnar cells. Examination of the midgut of the normal (control) sixth in star larva, showed the presence of cytoplasmic protrusions. The number of the protrusions present increased according to the age of the larva. In 24hour larva, these protrusions are limited to folds of the inner surface (Figs 9, 10). In the case of 48 hour age larva, protrusions spread onto all over the inner surface of the midgut (Figs 13,14). Fine brush border are seen in the midgut of control larva.

In the chromafenozide treated larval midgut, the cytoplasmic protrusions are also seen. In 24 hours larva there was no visible changes as when compared with the control (Figs 11, 12). Cytoplasmic protrusions and fine brush borders of the columnar cells are present as in the control larva. In the case of 48 hour old larval midgut, degradation of the cytoplasmic protrusions has commenced and the brush border of the columnar cells is completely destroyed. They were more or less absent in some regions (Figs 15, 16).

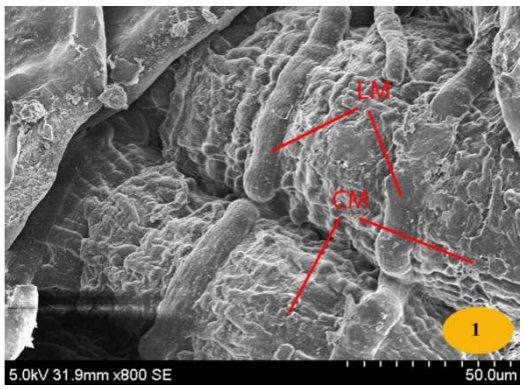

Fig 1 Scanning Electron Micrograph of midgut of *S.mauritia* control (24 hr) outer surface. Tightly packed longitudinal muscle bundle (LM) and circular muscle bundles (CM) (50µm)

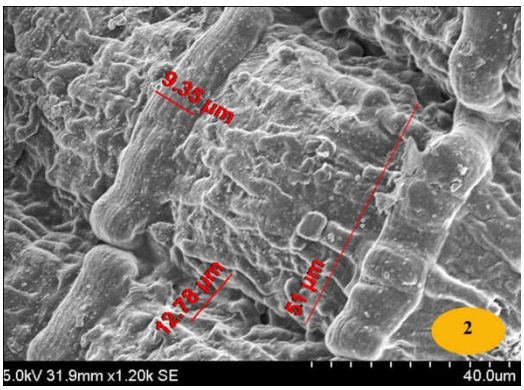

Fig 2 SEM image of *S.mauritia* control (24 hr) outer surface with morphometric measurements.(Width of LM, Width of CM and space between LM and CM) (40µm)

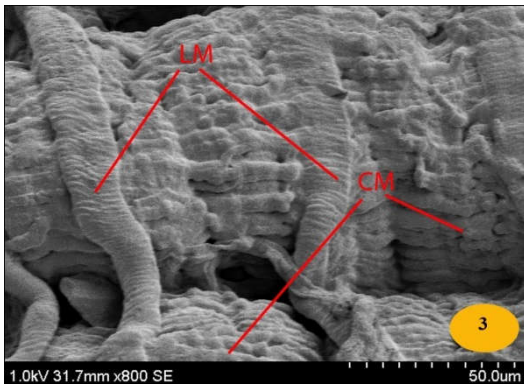

Fig 3 Scanning Electron Micrograph of midgut of *S.mauritia* treated with Chromafenozide (24 hr), showing outer surface with small depression in between circular muscle bundles (CM) (50µm)

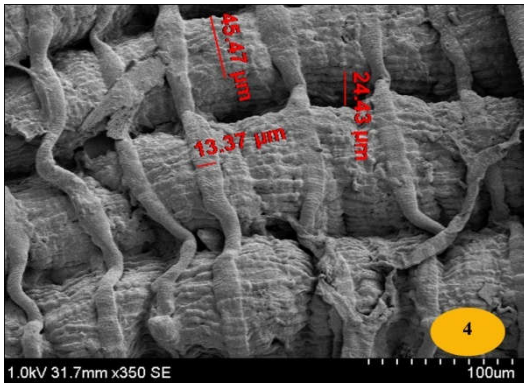

Fig 3 Scanning Electron Micrograph of midgut of *S.mauritia* treated with Chromafenozide (24 hr), showing outer surface with small depression in between circular muscle bundles (CM) (50µm)

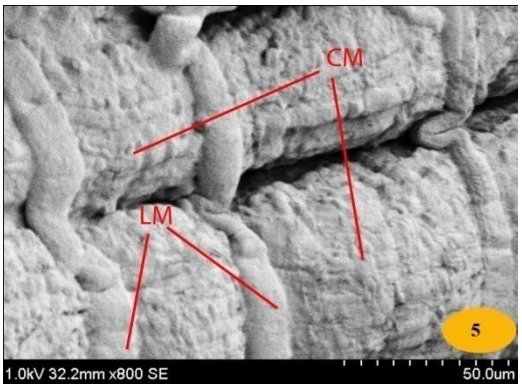

Fig 5 Outer surface of (48 hr) control midgut showing tightly packed longitudinal muscle bundles (LM) and circular muscle bundles (CM). (50µm)

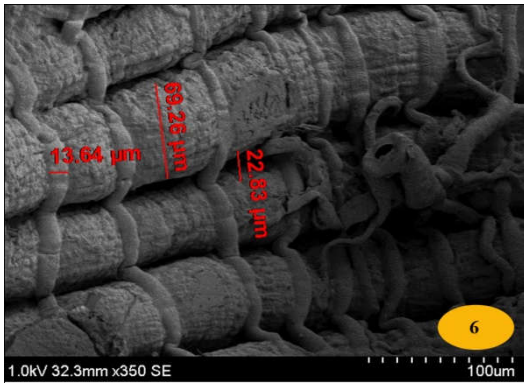

Fig 6 Outer surface of midgut (48 hr) with morphometric measurements. (Width of LM, width of CM and space between LM and CM) (100µm)

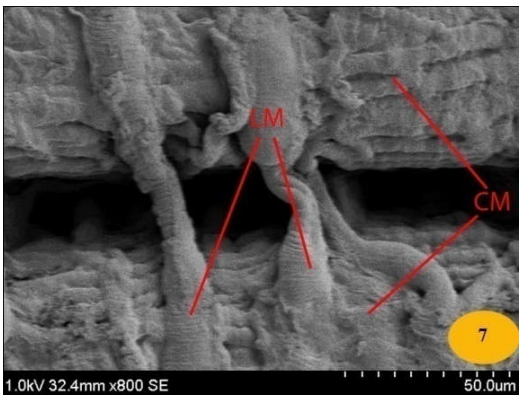

Fig 7 The outer surface of midgut treated after (48 hr). The muscle bundles are separated by deep depression due to degradation. (50µm)

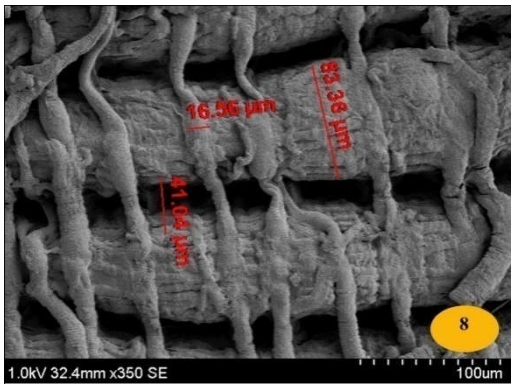

Fig 8 SEM image of Chromafenozide treated (48 hr) midgut outer surface (100µm) with morphometric measurements. (Width of LM, width of CM and space between LM and CM).(1000µm)

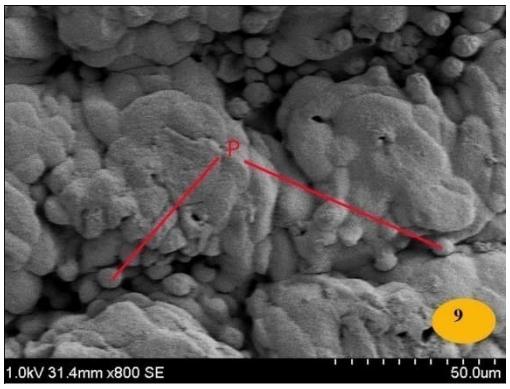

Fig 9 Inner surface of midgut (24 hr) control with less number of protrusions (P).(50µm)

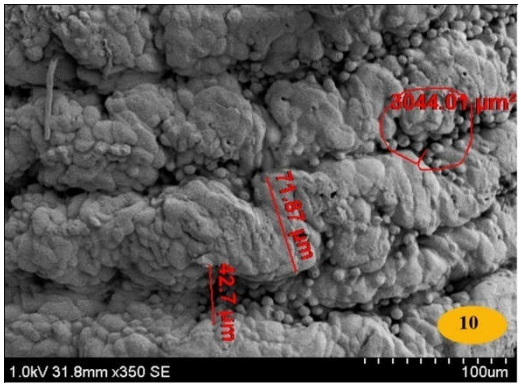

Fig 10 Inner surface (100µm) of control (24 hr) with morphometric measurements (Width of the tissue fold, space between two tissue folds and protrusions present in an area). (100µm)

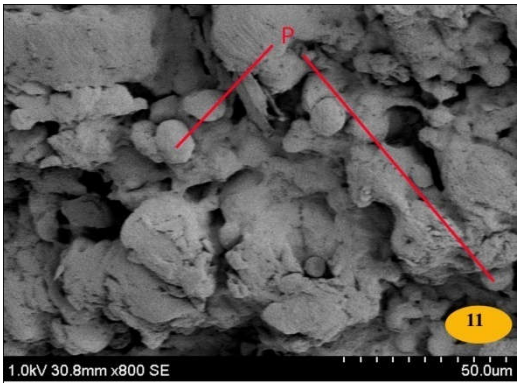

Fig 11 Chromafenozide treated (24 hr) midgut inner surface with several numbers of cytoplasmic protrusions (P). (500µm)

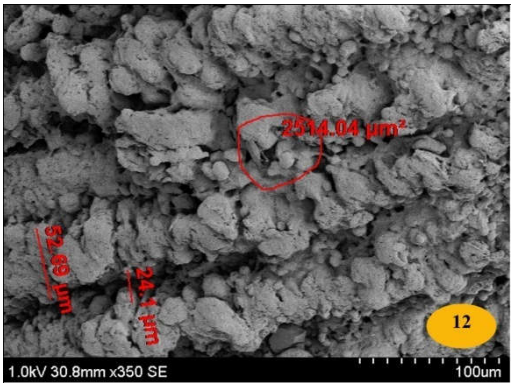

Fig 12 SEM image of Chromafenozide treated (24 hr) *S.mauritia* midgut inner surface with morphometric measurements (width of the tissue fold, space between two tissue folds and protrusions present in a particular area). (100µm)

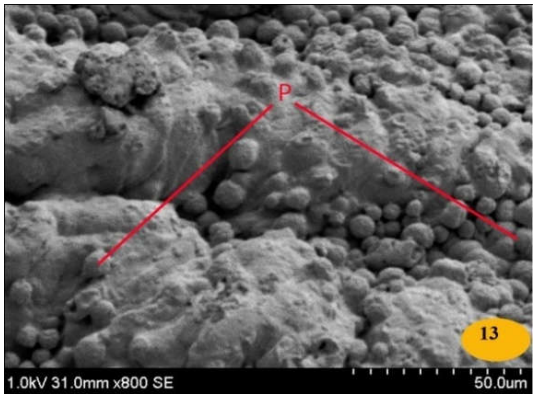

Fig 13 Inner surface (50µm) of (48 hr) control with more numbers of protrusions (P). (50µm)

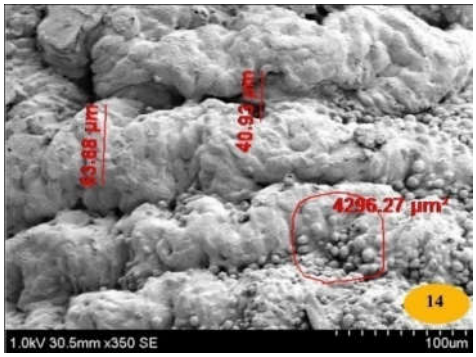

Fig 14 SEM image of control (48 hr) *S.mauritia* midgut inner surface with morphometric measurements (width of the tissue fold, space between two tissue folds and protrusions present in a particular area) (100µm)

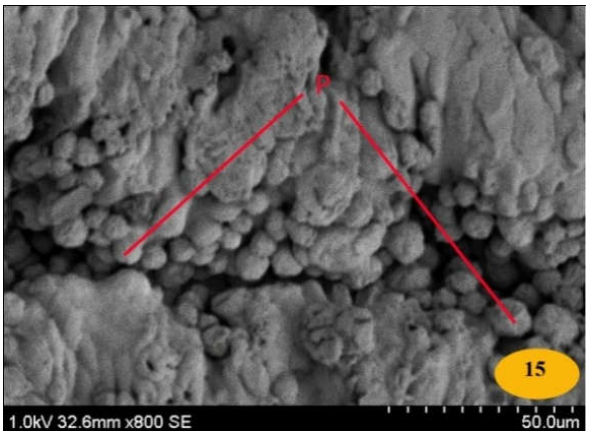

Fig 15 Treated (24 hr) midgut inner surface with more number of degraded protrusions (P). (50µm)

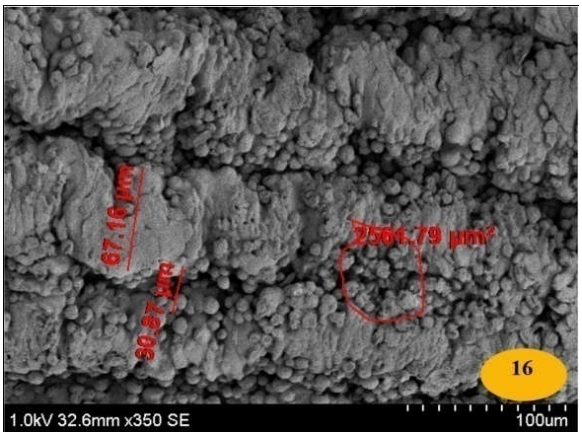

Fig 16 SEM image of Chromafenozide (48 hr) *S.mauritia* midgut inner surface with morphometric measurements (width of the tissue fold, space between two tissue folds and protrusions present in a particular area). (100µm)

Morphometric measurements of the structures of outer and inner surfaces of the midgut

In the outer surface of the midgut longitudinal muscle bundles and circular muscle bundles are the main structures present. Both of them give exact structure to the midgut. As described above some degree of changes are observed in the muscular layers due to the treatment of the compound. To measure the intensity of this, the width of both muscle bundles and the space between the two circular muscles bundles were examined.

The effects of chromafenozide treatment on the midgut morphology of sixth instar larvae of *S.mauritia* are depicted in Table 1. The data obtained reveals that the width of longitudinal bundles of control larvae of day 1 (24hr age) and that of the treated larvae showed no significant change. The width of longitudinal muscles of control larvae is 11.048±2.264µm and that of treated larvae is 11.483±2.027µm. But in the case of day2 (48hr age), larvae exhibited a slight reduction in the width of longitudinal muscles of treated larvae. The width of longitudinal bundle of control larvae is 14.104±2.125µm and that of treated larvae is 13.017±2.959µm. The width of the circular bundle of control 24hour age larvae is 57.159±7.930µm and that of treated larva is 50.158±10.088µm.

Table 1 Morphometric measurements of outer surface of midgut

Mid gut samples	Width of Longitudinal muscle bundle (µm)	Width of circular muscle bundles (µm)	Space between circular muscle bundles (µm)
Control (24hr)	11.048±2.264 (0.413)	57.159±7.930 (1.447)	8.926±1.858 (0.339)
Treated (24hr)	11.483±2.027 (2.959)	50.158±10.088 (1.841)	8.550±1.730 (0.315)
Control (48hr)	14.104±2.125 (0.388)	75.452±5.925 (1.081)	16.654±3.771 (0.688)
Treated (48hr)	13.073±2.959 (0.379)	80.2107±6.446 (1.770)	23.035±2.826 (0.515)

Values are expressed as mean ± standard deviation (standard error of mean)

At the same time, width of circular muscle bundle of control 48hour larva is 75.452±5.925µm and the same of treated is 80.127±6.446µm respectively.

The results of the measurements of the space between the circular muscle bundles shows that significant degradation occur in between the circular muscle bundles as a result of the effect of compound. In 24hour after the treatment, there was no significant change in the space in both control and treated larvae and the bundles are closely packed. But after 48 hour, the space between them increases

The data illustrated in (Table 2) shows the measurements of the inner surface of the midgut. In the inner surface of the midgut, tissues are arranged in folding. The width of these tissue folds decreases from day1 (24hr) to day2 (48hr). But in treated individuals the width considerably decreases as when compared to that of control larva. In 48hour, the width increased up to 64.326±15.112µm. The space between the tissues folds also had some significant changes. It is observed that the space between the tissue folds decreases in 48 hour control and treated larva.

Table 2 Morphometric measurements of inner surface of the midgut

Mid gut samples	Width of the tissue fold (µm)	Space in between two tissue folder (µm)	Number of protrusions present in 500µ^2
Control (24hr)	56.751±3.554 (1.689)	32.521±10.226 (2.286)	4.696±1.750 (0.553)
Treated (24hr)	53.417±14.316 (3.201)	27.232±9.209 (2.059)	5.657±1.851 (0585)
Control (48hr)	39.921±13.356 (2.986)	28.689±3.934 (0.879)	2.120±0.621 (0.196)
Treated (48hr)	64.326±15.112 (3.379)	29.161±7.333 (1.639)	4.656±1.441 (0.455)

Values are expressed as Mean ± Standard deviation (standard error of mean)

The other significant observation of the present study evidenced was on the number of cytoplasmic protrusions of columnar cells of the midgut. Their number in 500µ^2 area was counted and from the results obtained it is evident that there is an increase in the number (Table.2). In control (24hr), 4 protrusions are present, but in treated it increased up to 5. However, in 48 hour age control larva, 2 protrusions are present where as in treated one, 4 numbers were evident.

DISCUSSION

Midgut of an insect is an important part where the digestion and intake of nutrients takes place. Histological observation on last instar larvae of *S. mauritia* revealed that the midgut morphology was intact in position with epithelial layer, and outer layer of longitudinal muscles. The general morphology of midgut epithelium of *S.mauritia* larva is similar to that described for many lepidopterans, such as *Manducasexta* (Cioffi, 1979), *S. frugiperda* (Jordão*et al*., 1999), *Anticarsiagemmatalis* Hübner (Levy *et al*., 2004) and in *Diatraeasaccharalis*. F (Daniela *et al*., 2008).

The outer and inner surface of the midgut were examined under scanning electron microscope and photographed. The existing knowledge on the ultrastructural aspects of midgut is comparatively very less. Examination on the ultramorphology of midgut outer surface showed two types of muscle bundles longitudinal and circular. Similar observations are recorded in *S .frugiperda* Smith (Jordão *et al*. 1999) *Anticarsiagemmatalis* Hübner (Levy *et al*., 2004) and in *Diatraeasaccharalis*. F (Daniela *et al*., 2008). When the obtained data are morphometrically measured, the width of the longitudinal muscle bundles was reduced in 48hour treated larva as when compared to that of control. But in the case of the circular muscle bundles enlarged drastically. Also the space between the two circular muscle bundles also increased. This may be due to the effect of the compound on the outer surface of the midgut.

In inner surface also significant change in the tissue fold width are noticed. The tissue fold firstly reduced its size and then it enlarged drastically. Another interesting feature of the inner surface of the gut is the presence of numerous cytoplasmic protrusions. Similar feature are evidenced in *Diatraeasaccharalis*. F (Daniela *et al*., 2008). However, treatment of the compound in the larva showed a reduction in the number of cytoplasmic protrusions.

CONCLUSION

From the present observations, it is clearly evidenced that the treatments of ecdysone agonist, chromafenozide have caused detrimental morphological aberrations on the midgut of *Spodopteramauritia*. Moreover, the intensity of the ultra-morphological aberrations of muscular areas was higher in the treated samples.

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References

1. Oberlander H, Silhacek D L, Porcheron P. Nonsteroidal ecdysteroid agonists: tools for the study of hormonal action. *Archives of Insect Biochemistry and Physiology* 1995; 28: 209-223.

2. Dhadialla T S, Carlson G R, Le D P. New insecticides with ecdysteroidal and juvenile hormone activity. *Annual Review of Entomology* 1998; 43: 545-569.
3. Nakagawa Y. Non steroidal ecdysone agonists. Vitam. *Horm*. 2005; 73: 131-173.
4. Mordue (Luntz) A J, Nisbet A J.Azadirachtin from the neem tree *Azadirachtaindica*: its actions against insects. Anais da Sociedade Entomologica do Brasil. 2000; 29 (4): 615-632.
5. Ephrussi B, Beadle G W. A technique of transplantation for Drosophila. *Amer. Nat*, 1936; 70:218-225.
6. Cioffi M. The morphology and fine structure of the larval midgut of a moth (*Manducasexta*) in relation to active ion transport. *Tissue Cell* 1979; 11: 467-479.
7. Jordão B P, Capella A N, Terra W R, Ribeiro A F, Ferreira C. Nature of the anchors of membrane-bound aminopeptidase, amylase, and trypsin and secretory mechanisms in *Spodopterafrugiperda* (Lepidoptera) midgut cells. *Journal Insect Physiology Oxford*, 1999; 45: 29-37.
8. Levy S M, Falleiros A M F, Moscardi F, Gregorio G G, Toledo. Morphological study of the hindgut in larvae of *Anticarsiagemmatalis*Hubner. (Lepidoptera: Noctuidae). *Neotrp. Entomol* 2004; 33: 427- 431.
9. Daniela O, Pinheiro Quagio-Grassiotto I, Gegório E A. Morphological Regional Differences of Epithelial Cells along the Midgut in Diatraeasaccharalis Fabricius (Lepidoptera: Crambidae) Larvae. *Neotropical Entomology* 2008; 37(4):413-419.

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