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HISTOPATHOLOGICAL EFFECTS OF TWO INSECT GROWTH REGULATORS ON THE MIDGUT OF THE THIRD LARVAL INSTAR OF *CEPHALOPINA TITILLATOR* (DIPTERA: OESTRIDAE)

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ABSTRACT

The effects of LC_{50} of two insect growth regulators (IGRs), pyriproxyfen and chlorfluazuron, on the histology of the midgut of the 3rd instar larvae of the camel nasal bot fly, *Cephalopina titillator*, were studied. This study indicated that the anterior and posterior parts of the midgut were highly affected after 24 and 48h post-treatment. The effect of LC_{50} of pyriproxyfen on the midgut revealed that the anterior part was highly affected than the posterior part, as it had a complete destructive effect on the muscular layer and basement membrane. The epithelial layer turned dark in colour, whereas both the cell boundaries and nuclei disappeared.

In contrast to the effect of pyriproxyfen, chlorfluazuron caused less destruction in the anterior midgut region of the larvae than the posterior region at 24 and 48h post-treatment. 24h post-treatment, the histological structure of the posterior midgut of larvae treated with chlorfluazuron showed loosely attached epithelial layer in sections which became filled with vacuoles in 48h post-treatment sections. The muscular layer was dissolute, the peritrophic membrane could not be detected and the lumen had masses of cellular material in sections, both 24 and 48h post-treatment. Also, chlorfluazuron was found to have minimal effects on the midgut of treated larvae compared to pyriproxyfen.

INTRODUCTION

Larvae of *Cephalopina titillator* are obligate parasites on the camel and are regarded as the sole cause of nasopharyngeal myiasis in the host (Zumpt, 1965).

Most of the previous studies on this parasitic insect were based mainly on the morphology, incidence of a disease, biological aspects, prevalence, monthly variations and few methods of control (Hussein *et al.*, 1983; Desbordes and Ajogi, 1993; Fatani and Hilali, 1994; Ajogi and Desbordes, 1995 a and b; Morsy *et al.*, 1998; Nwosu and Wachy, 1998 and Alahmed, 2002).

Williams (1956) was the first to observe the blocking of adult differentiation when the juvenile hormone (JH) extracted from the moth, *Hyalophora cereopia* was injected into, or topically applied to young lepidopterous pupae. Since then, hundreds of compounds like cyclic terpenes,

non-terpenoids and aromatic terpenoids with JH effects were synthesized and bioassayed on many insect species (Slama *et al.*, 1974; Quesada and Montoya, 1994; Rao *et al.*, 1994; Gasana *et al.*, 1999 and Chism and Apperson, 2003).

Previous work was undertaken to evaluate the effects of pyriproxyfen and chlorfluazuron on the protein level and RNA intensity in the 3rd instar larvae of *C. titillator* (El Bassiony *et al*., 2005). The latter authors stated that Chlorfluazuron was found to be more toxic against the 3rd instar larvae than pyriproxyfen, whereas the LC_{50} values were 310.07 and 967.53 ppm for chlorfluazuron and pyriproxyfen, respectively.

The present histopathological study aims to confirm and augment our previous biochemical and molecular results (El Bassiony *et al*., 2005), which were recorded in the insect under the effect of both studied IGRs.

MATERIALS AND METHODS

Third instar larvae of the camel nasal bot fly, *C. titillator*, were collected from several camels slaughtered in Cairo, Egypt as described by El Bassiony (2004).

Partial rearing of *C. titillator* larvae in the laboratory was carried out according to El-Moursy *et al.* (1993).

Pyriproxyfen and chlorfluazuron are insect growth regulators. Pyriproxyfen (S-71639) is a juvenile hormone analogue; whereas chlorfluazuron (IKI-7899) is a chitin synthesis inhibitor.

Healthy apparent 3rd instar larvae of *C. titillator* were topically treated along the dorsum of the larvae with LC_{50} of pyriproxyfen and chlorfluazuron with a dosage 5µl/ larva, using a micropipette (Socorex 841). Acetone-treated larvae served as a control group. The midgut was dissected, in insect saline, from living individuals of the three groups (acetone-treated group, larvae treated with LC_{50} of pyriproxyfen and larvae treated with LC_{50} of chlorfluazuron) after 24 and 48h post-treatment.

The midgut of the larvae of *C. titillator* is long, so it was divided into two parts (anterior and posterior midgut).

Histological preparations for the midgut of both control and treated larvae were prepared according to Disbrey and Rack (1970) by the double embedding procedure.

RESULTS and DISCUSSION

The morphological structure of the midgut or the mesenteron of the 3rd instar larvae of *C. titillator* showed that it is a long tube of various diameters folded around itself and around the hindgut. It begins as a wide tube, which turns to a transparent tube in the middle region and ends as a very narrow tubular structure of uniform diameter. Similar observations were previously reported by Fayed (2000) for the same fly.

1– Histology of control midgut of 3rd instar larvae of *C. titillator*

Histologically, no differences were recognized between the anterior and the posterior parts of the midgut of the control 3rd instar larvae of *C. titillator*. However, Fayed (2000) recognized histological differences between

the anterior part and the posterior part of midgut of the prepupal instar of *C. titillator*.

The midgut consists of an outer sheath of muscles with a thin and developed outer longitudinal muscle layer and an inner circular muscle layer. Internal to the inner circular muscle layer, a hardly apparent thin basement membrane with an epithelial layer is found. The uniformly arranged columnar cells have rounded nuclei (central and loaded with chromatin particles) and distinct cell boundaries. The apical surface of each columnar cell has an irregular striated border. This border may be hardly visible when the lumen of the midgut filled with the degenerate cellular debris and food remnants. Between the epithelial cells lie the regenerative cells (Fig 1, A&B).

2– Effect of pyriproxyfen treatment on the midgut of the 3rd instar larvae of *C. titillator*

In the present investigation, the effect of LC_{50} of pyriproxyfen on the anterior part of the midgut of *C. titillator* was more pronounced than on the posterior part.

The anterior midgut of the treated larvae, of both 24h (Fig 2) and 48h (Fig 3) post-treatment, showed complete destruction of the muscular layer and basement membrane. The epithelial layer turned dark in color, whereas both the cell boundaries and nuclei disappeared.

On the other hand, pyriproxyfen treatment affected the posterior part of the midgut of *C. titillator* in different ways. 24h post-treatment, the basement membrane and the muscular layer separated from the epithelial layer, and the longitudinal muscle layer was more or less degenerated (Fig 4, A&B). Also, the epithelium lost its columnar form, the cells became loosely attached to each other and had vacuolated nuclei (with little chromatin material). Similar findings were previously mentioned on *Aedes aegypti* larvae treated with pyriproxyfen (Syafruddin *et al*., 1990).

Sections of the posterior midgut, 48h post-treatment with pyriproxyfen (Fig 5, A&B), showed a thinner muscular layer than that of the posterior midgut 24h post-treatment. Also, cytoplasmic extrusions of degenerated cells and disappearance of some cell nuclei were noticed. The peritrophic membrane was not only separated from the epithelial layer, as in 24h post-treatment, but was also disrupted.

Patel and Madhaban (1969) discussed the changes in the mitotic activity, of the midgut cells, which were induced by additional juvenile factor, and reported that these changes were alterations in the rate of RNA and protein synthesis. It appears, therefore, that the histopathological changes occurring in the midgut of *C. titillator* treated with the juvenile hormone analogue (pyriproxyfen) in the present study, account for the decrease in the rate of mitosis in the epithelial cells of the midgut.

The increase in histological malformation, which was detected in 48h post-treated tissues of midgut of *C. titillator,* than that of 24h post-treated ones may be due to the rapid cuticular penetration of pyriproxyfen and relatively large proportions of the applied concentration which accumulated internally because of the slow metabolism and excretion of the IGR (Bull & Meola, 1993).

3- Effect of chlorfluazuron treatment on the midgut of the 3rd instar larvae of *C. titillator*

In contrast to the effect of pyriproxyfen, chlorfluazuron treatment caused less destruction at 24 and 48h post-treatment in the anterior midgut of 3rd instar larvae of *C. titillator* than in the posterior region.

The major alterations found in the anterior midgut of *C. titillator* treated with chlorfluazuron, in the present work, were detected in the 24h post-treatment sections, as the epithelial cells became less in number, cell-boundaries began to dissolve and some cell nuclei disappeared (Fig 6, A&B). These changes became more severe in 48h post-treatment sections, where the cells were partially dissolved, vacuolated, became swollen, most of the cell-boundaries dissolved and some cells moved towards the gut lumen (Fig 7, A&B). In 24h post-treatment sections, the lumen was filled with masses of degenerative cell remnants and cellular debris. Also, the muscular layer was thinner than that of control sections with scattered, weakly developed longitudinal muscle bundles. These bundles were hardly detected in 48h post-treatment sections.

Similar observations occurred in several insects, treated with several IGRs and plant extracts. Schluter (1986) reported that the azadirachtin treated *Manduca sexta* larvae caused supernumerary moult between the 5th and 6th larval instars as the epithelium was degenerated. Also, Hamouda *et al*. (1996) treated the 3rd larval instar of *Culex pipiens* with various fractions of *Artemisia judaica* and *Anagallis arvensis* mixed with water. They found that the midgut of larvae treated with *A. judaica* was affected as the epithelial layer was vacuolated, had swollen cells, masses of cellular material appeared in the anterior part of the midgut and finally the epithelium lost its normal appearance. On the other hand, the same authors indicated that the larvae treated with *A. arvensis* showed rupture of the cell wall and destruction of peritrophic membrane. Furthermore, Younes *et al*. (2000) recorded that capillin isolated from *Artemisia monoperma* elicited severe histopathological effects on the structure of the midgut of larvae of *Tribolium castaneum*.

The histological structure of the posterior midgut of *C. titillator* 24h post-treatment with chlorfluazuron showed a loosely attached epithelial layer (Fig 8, A&B) and this became filled with vacuoles in sections of 48h post-treatment larvae (Fig 9, A&B). The muscular layer was dissolute, the peritrophic membrane could not be detected and the lumen had masses of cellular material in sections of both 24 and 48h post-treatment larvae. These results run in agreement with the previous observations of Pelsue (1985), who studied the effect of diflubenzuron and triflumuron (chitin inhibitors) on the alimentary canal of *Chironomus* larvae, where the cells appeared lacy or reticulate and containing large vacuoles.

The malformation in the midgut of larvae treated with chlorfluazuron was less than that treated with pyriproxyfen. These findings are in agreement with those of El Bassiony *et al*. (2005).

In view of the above considerations, the histological alterations obtained, in the present study, in the midgut of the 3rd instar larvae of *C. titillator* treated with pyriproxyfen and chlorfluazuron may be due to the interference of these IGRs with cell division, or the endocrine system or both (Patel & Madhaban, 1969; Shultz, 1985).

Results suggested that pyriproxyfen is more effective in controlling *C. titillator*. Also, the two studied IGRs, pyriproxyfen and chlorfluazuron, may be recommended to be used for the control of the insect, after studying their toxicity to the insect host (camel), because they caused destructive effects on different biochemical (El Bassiony *et al*., 2005) and histological (present study) components of one important instar of this insect.

REFERENCES

Ajogi, I. and Desbordes, C.K. (1995a). Seasonal prevalence of *Cephalopina titillator* myiasis in camels (*Camelus dromedarius*) in Sokoto State, Nigeria. Bull. Anim. Health Prod. Africa, **43**: 295-296.

Ajogi, I. and Desbordes, C.K. (1995b). Seasonal prevalence of *Cephalopina titillator* myiasis in camels (*Camelus dromedarius*) in Sokoto State, Nigeria. Israel J. Vet. Med., **50(2)**: 67-69.

Al Ahmad, A.M. (2002). Seasonal prevalence of *Cephalopina titillator* larvae in camels in Riyadh region, Saudi Arabia. Arab-Gulf J. Scient. Res., **20 (3)**: 161-164.

Bull, D.L. and Meola, R.W. (1993). Effect and fate of the insect growth regulator pyriproxyfen after application to the horn fly (Diptera: Muscidea). J. Econ. Entomol., **86**: 1754-1760.

Chism, B.D. and Apperson, C.S. (2003). Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. Med. Vet. Entomol., **17 (2)**: 211-220.

Desbordes and Ajogi (1993). Seasonal prevalence of *Cephalopina titillator* myiasis in camels (*Camelus dromedarius*) in Sokoto State, Nigeria. Vet. Parasitol., **50**: 161-164.

Disbrey, B.D. and J.H. Rack (1970). Histological Laboratory Methods. E. and S. Livingstone, Edinburgh and London.

El Bassiony, G.M. (2004). Karyotype and nucleic acid patterns of the camel nasal bot fly *Cephalopina titillator* (Clark) (Diptera: Oestridae). Bull. Ent. Soc. Egypt, **81**: 129-139.

El Bassiony, G.M.; Dorrah, M.A. and El Nady, A.M. (2005). Effects of two insect growth regulators on the protein level and RNA intensity in the larvae of the camel nasal bot fly, *Cephalopina titillator* (Clark, 1797). Bull. Entomol. Soc. Egypt, **82**: 1- .

El-Moursy, A.A.; Madbouly, M.H.; Abdel Meguid, A.; Zayed, A.A. and El-Khateeb, R.M. (1993). Longevity of *Cephalopina titillator* (Diptera: Oestridae) adult fly at different laboratory conditions. J. Egypt. Vet. Med. Ass., **53 (4)**: 649-652.

Fatani, A. and Hilali, M. (1994). Prevalence and monthly variations of the second and third instars of *Cephalopina titillator* (Diptera: oestridae) infesting camels (*Camelus dromedarius*) in the eastern province of Saudi Arabia. Vet. Parasitol., **53 (1-2)**: 145-151.

Fayed, H.A. (2000). Cytological studies on the midgut of the last larval stage of the nasal bot fly *Cephalopina titillator* (Oestridae: Diptera). M.Sc. Thesis, Fac. Sci., Helwan University, Egypt.

Gasana, G.V.; Gandia, B.A.; Mengodpuerta, C.; Primo, M.J. and Primo, Y.E. (1999). Insect growth regulators as chemosterilants for *Ceratitis capitata* (Diptera: Tephritidae). J. Econ. Entomol., **92 (2)**: 303-308.

Hamouda, I.S.; Elyassaki, W.M. and Hamed, M.S. (1996). Toxicity and histopathological effects of *Artemisia judaica* and *Anagallis arvensis* extracts on *Culex pipens* larvae. J. Egypt. Ger. Soc. Zool., **20 (E)**: 43-60.

Hussein, M.F.; Hassan, H.A.R.; Bilal, H.K.; Younis S.M.; Al-Motlaq, A.A.R. and Al-Sheikh, M.A. (1983). *Cephalopina titillator* (Clark, 1797) infection in Saudi Arabian camels. Zentralblatt F. Vet. Med., **30 (8)**: 553-558.

Morsy, T.A.; Abdel-Aziz, A.S.; Mazyad, S.A.M.; Al-Sharif, K.O. (1998). Myiasis caused by *Cephalopina titillator* (Clark) in slaughtered camels in Al Arish abattor, North Sinai Governorate. J. Egypt. Soc. Parasitol. **28(1)**: 67-73.

Nwosu, C.O. and Wachy, C.S. (1998). Prevalence of nasal myiasis in slaughtered camels (*Camelus dromedarius*) in the arid and semi-arid regions of Nigeria. J. Camel Pract. Res., **5 (1)**: 170-172.

Patel, N. and Madhaban, K. (1969). Effects of hormones on RNA and protein synthesis in the imaginal discs of the *Ricini* silkworm. J. Insect Physiol., **15**: 2141-2150.

Pelsue, F.W. (1985). Histopathological effects of two insect chitin inhibitors in the alimentary canal of chironomid midges (Diptera: Chironomidae). Bull. Soc. Vector Ecol., **10 (2)**: 72-89.

Quesada, B. and Montoya, L. (1994). Laboratory evaluation of chlorfluazuron against larval Phlebotomine sand flies (Diptera: Psychodidae). J. Econ. Entomol., **87 (5)**: 1129-1132.

Rao, N.V.; Rao, K. and Reddy, A.S. (1994). A note on the efficacy of IGR to caterpillar, *Helicoverpa armigera* (Hub.). J. Insect. Sci., **5**: 169-171.

Schluter, U. (1986). Effects of azadirachtin on developing tissues of various insect larvae. Proc. 3rd Int. Neem. Conf., 331-348.

Shultz, W.D. (1985). Histological investigations on the effect of seed extracts from the neem tree *Azadirachta indica* on female imagines of *Epilachna varinestis* (*Coleoptera*: *Coccinellidae*). 1- Destruction of ovarian development and oogenesis. Zool. Jahrb. (Anat. Ontog. Tiere.), **11**: 193-214.

Slama, K.; Romanuk, M. and Sorm, F. (1974). Insect hormones and bioanalogues. Springer Verlag, New York, Wien, 477 pp.

Syafruddin, S.; Arakawa, R.; Kammura, K. and Kawamoto, F. (1990). Histopathological effect of an insect growth regulator, 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether (pyriproxyfen) on the larva of *Aedes aegypti* 930-01, Japan. Jpn. J. Sanit. Zool., **41 (1)**: 15-22.

Williams, C.M. (1956). The juvenile hormone of insects. Nature, **178**: 212-213.

Younes, M.W.F.; Ryan, M.F.; Assar, A.A. and Sakr, H.H. (2000). Histopathological effect of Capillin (1-phenyl 1-2,4-hexadiyn-1-one) isolated from *Artemisia monosperma* on the integument and the midgut of the red rust flour beetle, *Tribolium castaneum* (*Coleoptera*: *Tenebrionidae*). Proc. 1st Int. Conf. Appl. Entomol., Cairo, pp. 145-167.

Zumpt, F. (1965). Myiasis in man and animals in the oldworld: A textbook for physicians, veterinarians and zoologists. Butter Worths, London.

EXPLANATION OF FIGURES

Bar in all figures = 50 µm.

Fig. (1, A-B): A: Photomicrograph of a transverse section in the anterior midgut of the control 3rd instar larvae of *C. titillator.*

B: Photomicrograph of a transverse section in the posterior midgut of the control 3rd instar larvae of *C. titillator.*

Fig. (2): Photomicrograph of a transverse section in the anterior midgut of the 3rd instar larvae of *C. titillator* 24h post-treatment with pyriproxyfen.

Fig. (3): Photomicrograph of a transverse section in the anterior midgut of the 3rd instar larvae of *C. titillator* 48h post-treatment with pyriproxyfen.

Fig. (2) and Fig. (3) show complete degeneration of the muscular layer and basement membrane. The epithelial layer turned dark in color, whereas both the cell boundaries and nuclei disappeared.

Fig. (4, A-B): Photomicrograph of a transverse section in the posterior midgut of the 3rd instar larvae of *C. titillator* 24h post-treatment with pyriproxyfen.

A: The basement membrane and the muscular layer were separated from the epithelial layer and the longitudinal muscle layer was more or less degenerated. **B:** Epithelium lost its columnar shape, became loosely attached to each other and had vacuolated nuclei (with little chromatin material).

Fig. (5, A-B): Photomicrograph of a transverse section in the posterior midgut of the 3rd instar larvae of *C. titillator* 48h post-treatment with pyriproxyfen.

A: shows thinner muscular layer than that of the posterior midgut 24h post-treatment, cytoplasmic extrusions of degenerated cells (arrow), disappearance of some cell nuclei and disruption of the peritrophic membrane. **B:** The basement membrane and the muscular layer separated from the epithelial layer and the longitudinal muscle layer was more or less degenerated.

Fig. (6, A-B): Photomicrograph of a transverse section in the anterior midgut of the 3rd instar larvae of *C. titillator* 24h post-treatment with chlorfluazuron.

A: The epithelial cells became less in number than in control sections and the lumen was filled with masses of degenerative cell remnants and cellular debris. **B:** The muscular layer was thinner than that of control sections with scattered and weakly developed longitudinal muscle bundles. Cell-boundaries began to dissolve and some cells nuclei disappeared (arrow).

Fig. (7, A-B): Photomicrograph of a transverse section in the anterior midgut of the 3rd instar larvae of *C. titillator* 48h post-treatment with chlorfluazuron.

A and **B:** Show that the cells were partially dissolved, vacuolated, became swollen, most of the cell-boundaries dissolved and some cells moved towards the gut lumen.

Fig. (8, A-B): Photomicrograph of a transverse section in the posterior midgut of the 3rd instar larvae of *C. titillator* 24h post-treatment with chlorfluazuron.

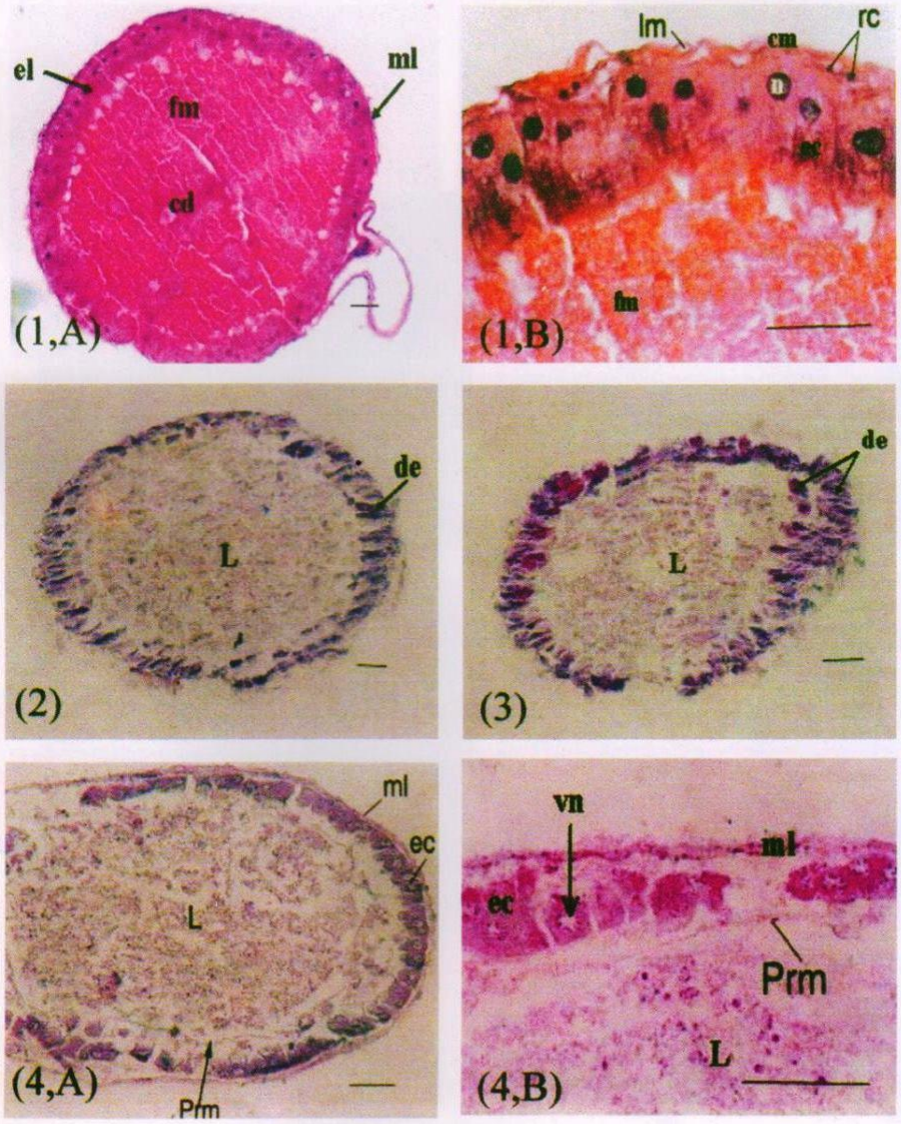
A and **B:** Show that epithelial layer became loosely attached, the muscular layer was dissolute, the peritrophic membrane could not be detected and the lumen had masses of cellular material.

Fig. (9, A-B): Photomicrograph of a transverse section in the posterior midgut of the 3rd instar larvae of *C. titillator* 48h post-treatment with chlorfluazuron.

A and **B:** Show loosely attached epithelial layer, which became filled with vacuoles. Also, the muscular layer was dissolute and the peritrophic membrane could not be detected.

LIST OF ABBREVIATIONS

Cellular debris(cd)
Circular muscle layer (cm)
Destroyed epithelium (de)
Epithelial cell (ec)
Epithelial layer (el)
Food mass (fm)
Longitudinal muscle layer (lm)
Luman (L)
Muscular layer (ml)
Nucleus (n)
Peritrophic membrane (prm)
Regenerative cells (rc)
Striated border (sb)
Vacuolated nucleus (vn)
Vacuoles (v)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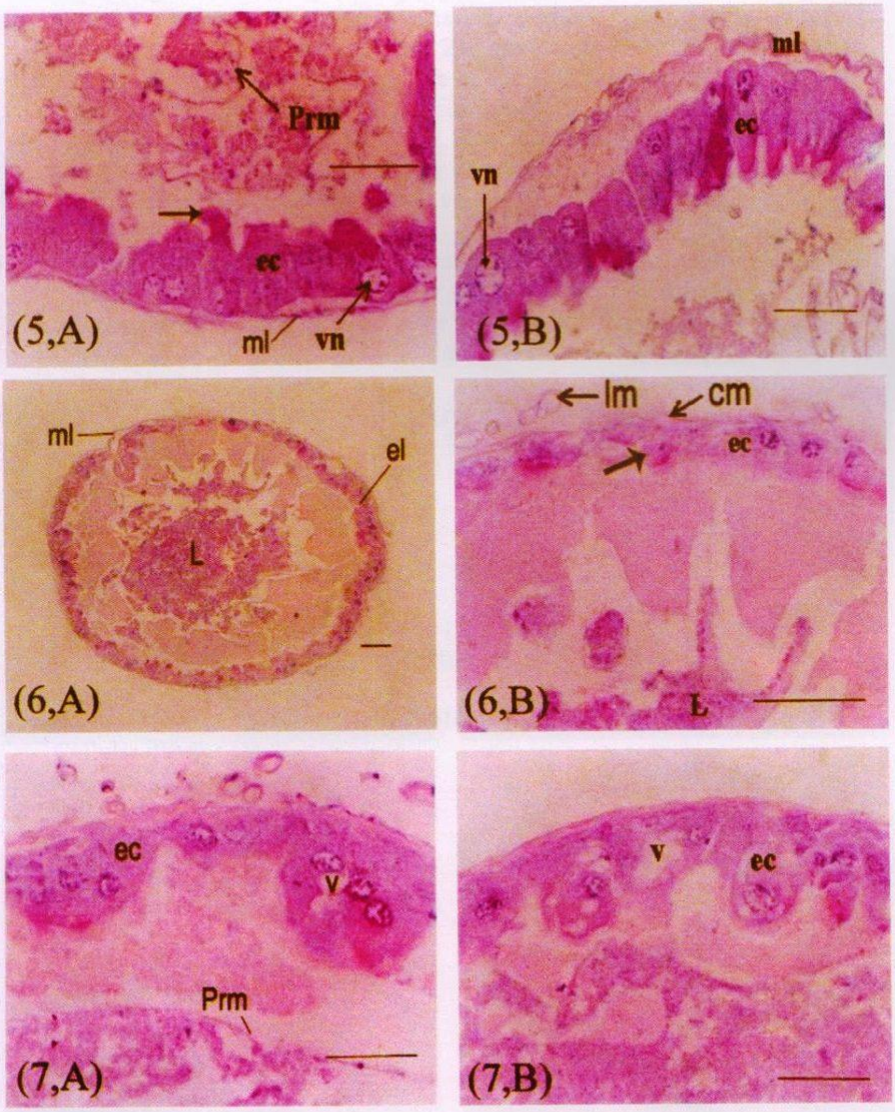
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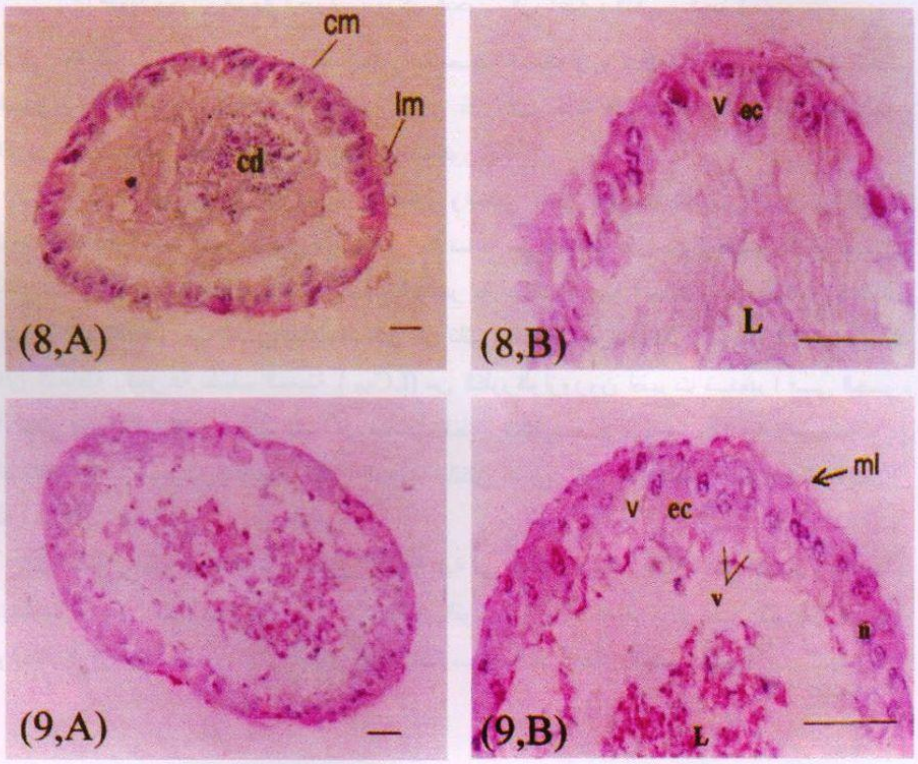
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