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# MOLECULAR CHARACTERIZATION AND EFFICACY OF *SPODOPTERA EXIGUA NUCLEAR POLYHEDROSIS VIRUS*

## 2- Anatomical and scanning changes in *Spodoptera exigua* (Hubner.) infected with *S.exigua nuclear polyhedrosis virus* Egyptian isolate (*SeNPV*)

[37]

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## ABSTRACT

***The*** beet army worm *Spodoptera exigua* (Hubner.) is affected by *nuclear polyhedrosis virus*, the disease specific polyhedral virus particles was found to be associated with the beet army worm (BAW). Inoculation of *SeNPV* into *S.exigua* induced characteristic symptoms of slow motion larvae, cuticle showing red color and swilling of the body. Examination of BAW infected extract by light microscope showed polyhedral bodies with concentration ($2.3x10^7$PIB/ml). In our study the morphological structures of *S.exigua* infected with *SeNPV* (EG) as well as healthy one were examined by SEM. It was showed complete destruction of external larval body, reduction in each abdominal segment size, pro-legs and thoracic legs reduce in size and appear short and stumpy also the oval shape spiracle converted to circular shape with rupture and disrupted inside tissue compared to healthy one. Healthy and infected *S.exigua* midgut epithelial cells histological structure was checked by using light microscope. It was found that the midgut epithelial cells were the first targets of the virus infection. Columnar cells, goblet cells and regenerated cells loose their identity and become disorganized also breaking down of peritrophic membrane and basement membrane and scatter of viral polyhedra and small empty vacuoles were observed.

**Key words:** *Spodoptera exigua*, *Nuclear polyhedrosis virus*, Morphological and Histological characterization.

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## INTRODUCTION

The beet armyworm (BAW). *Spodoptera exigua* (Habner.) is a widely distributed and highly destructive noctuid pest that is both difficult and costly to control with currently registered chemicals insecticides **(Trumble and Baker, 1984)**. Attempts to develop more effective control agents have included evaluation of *SeNPV* (Baculoviridae).The family Baculoviridae consists of enveloped invertebrate pathogenic viruses containing a circular double stranded DNA genome ranging from 90 to 160Kb **(Blissard and Rohrman, 1990)**. The virus enters its hosts through midgut epithelial cells from where infection spread throughout the insect. One characteristic of this virus is the production of two different structural forms that have distinct roles during infection of the host organism, occlusion derived virus (ODV), needed to spread the infection between the larvae and the budded virus (BV), needed for the dissemination of the infection within the host. Infection of the larval midgut is the prerequisite for successful baculovirus infection **(Flipsen *et al.*, 1995)**. The life cycle of baculovirus starts with the ingestion of OBs present on contaminated diet by larvae **(Kikhno *et al.*, 2002)**. The OBs are dissolved in the alkaline structure of the midgut and virus particles are released into gut lumen. After replication in the columnar epithelial cells, budded viruses are formed, which spread the infection to other susceptible tissues within the larval host **(Volkman and Keddie, 1990)**. The ODVs pass through the peritrophic membrane and infect the larval midgut cells which considered the first and principle site of the virus infection. The destruction of midgut epithelial cells proved the morphological abnormalities of external larval body. The present work aimed to study some morphological structure of *SeNPV* by using SEM as well as histological structure of their midgut epithelial cells by using light microscope.

## MATERIALS & METHODS

### I- Source of *SeNPV*:

The virus strain of *SeNPV* obtained from Virology Lab, Faculty of Agriculture, Ain shams university. **(Yasmin *et al.*, 2010)**.

### II- Morphological and Histological studies:-

### II-1- Insect rearing:

The beet army worm, *S.exigua* was obtained from Insect Pathogen

Unit (IPU) of Plant Protection Research Institute, Agricultural Research Center. The larvae were reared in the laboratory under highly controlled conditions to avoid contamination. Larvae fed on artificial diet described by **Shorey and Hale (1965).** The insect culture was maintained at 30°C, 70-80% relative humidity and a 16 h photoperiod **(Smits, 1987).**

**II-2- *S.exigua* infection by *SeNPV*-EG:**

*S.exigua* larvae were infected with *SeNPV*-EG by both the droplet-feeding method **(Hughes & Wood, 1981)** and diet surface treatment procedure **(Addy, 1969)** in which the larvae were starved for 16-20h and then allowed to drink from polyhedra which offered to the larvae as droplets of 5-10 µl applied in a circle into a layer of parafilm on the bottom of a petri dish After 15 min the larvae that had drunk from the suspension transfer to cups with contaminated artificial diet. The larvae were observed daily to identify the *NPV* infected ones based on the sign and symptoms of disease (loss of appetite, slow motion, easily injured skin and red color).

**II-3- Detection of occlusion bodies (OB):**

Occurrence of viral infection was proved with Giemsa staining. A thin smear of infected insect tissue slide was prepared. The smear was immersed for 1-2min in Giemsa, rinsed under running tap water for 5-10sec then the smear was stained for two hours in 10% Giemsa stain. After staining, the dye was rinsed off in running tap water for 5-10s and allowed to dry in air then examined under light microscope to detect the OBs **(Mustafa Yaman *at al.*, 2001).**

**II-4- Histopathological characterization:**

Samples of healthy and infected larvae (Third-instar larvae of *S. exigua* infected with *SeNPV* at different hours post inoculation started) were fixed in 10% neutral formalin, the samples were dehydrated in graded ethanol to xylene and embedded in paraffin. Sections were cut at a thickness of 4-5µm and stained using Haematoxylin/eosin for larval tissue according to **(Bancroft and Stevens (1996)).** The stained sections were observed and photographed using an optical microscope.

**II-5- Morphological features by scanning electron microscope:**

Samples of healthy and infected larvae were fixed in glutaraldehyde (2.5%) in 0.1 M phosphate buffer (0.1M $Na_2HPO_4$. $7H_2O$, 0.1M $KH_2O_4$ (PH 7.2)) for 24 h period at

4°C. Samples were post-fixed in osmium tetroxide (1% $OsO_4$) for one hour at room temperature **(Harley and Fergusen, 1990)**, then dehydrated with pathing through ascending concentrations of acetone and were dried till the critical point. Finally, samples were sputter coated with gold. The examination and photographing were done through **a Jeol Scanning Electron Microscope (T.330A) in the Central Laboratory of Faculty of Agriculture. Ain Shams University.**

## RESULTS

**Light microscope examination:**

Confirmation of infection of *S.exigua* by *SeNPV* by staining thin smear of infected larvae with Giemsa stain was done by Light microscope and proved the presence of viral occlusion bodies which appear clear and round particles.

**Midgut histological studies:**

The histological structure of the midgut which considered the largest part of the digestive tract of lepidopteran insects of untreated *S. exigua* showed as seen in **Figure (1)** that the midgut is lined with an epithelial layer, composed of a single layer of three types of cells which rest on a basement membrane. The majority of which were expressed by columnar cells which of prismatic form and show basophilic cytoplasm and elongated basal nucleus that occupies middle position within the cell and bears a striated or brush-like border (microvilli). Columnar cells are responsible for processing the diet, secretion of digestive enzymes and uptake of the final products. Also, several goblet cells were scattered randomly between the columnar cells, present a large cavity in the form of a calyx, whose internal surface contains projections similar to microvilli, they also contain U-shaped basal nucleus in the base of the chamber and acidophilic cytoplasm. The goblet cells co-operate with the columnar cells in ionic homeostasis and metabolite absorption. Regenerative cells were also detected, appear small in size with a round or elongated format, found singly, in pairs or in groups at the base of the epithelium, present central nucleus and basophilic cytoplasm.

The histopathological studies of fourth instar larvae treated with *SeNPV* after different hours of infection revealed the pathogenic effect of the virus on different

tissues. The infection begins after ingestion and when polyhedra are dissolved in alkaline medium of the midgut, hence, peritrophic membrane and midgut epithelial cells were the first targets of virus infection, the effect of virus on the midgut epithelial cells could be summarized as follows:-

1- Due to the activity of the virus there is hyper-proliferation of the epithelial cells make the peritrophic membrane appear voluminous lead to formation of finger like projection as a result of cytoplasmic protrusions **Figure (2 A)**.
2- The columnar cells loose their compact appearance and distended with disrupted microvilli. Also, in some areas the epithelial cells lose their close association with basement membrane, while in other areas the cell remain intact. The peritrophic and basement membrane appear broken down in some places **Figure (2 B).**
3- The midgut was occupied with many empty small vacuoles and the virus polyhedra were found scattered in different part of midgut, suggesting the spread of infection in almost all the tissues of the larvae **Figure (2 A, B)**
4- All the midgut cell (columnar, goblet and regenerative cells) either lost their identity or became highly disorganized.
5- In the final stage of infection there is complete destruction of all internal tissues of the midgut include basement and peritrophic membrane **Figure (2 C)**.

**Morphological features:**

The scanning surface technique was carried out on larvae of *S.exigua* infected with *SeNPV* as well as healthy ones. This study aim to cyto-pathic effects of *NPV* on *S.exigua* through Scanning electron microscope (SEM).

**Infected *S. exigua* :**

The morphological characters of *NPV* infected *S.exigua* larvae by using (SEM) showed abnormality revealed the destructive capacity of the virus to every part of the external larval body. The complete damage of all internal organs of the midgut epithelial cells as a result of virus infection reflect the shrinking of all external body segments specially at the midgut area make the external body surface (cuticle) appear flaccid, wasteful and completely damage compared to the control larval body which appear intact and healthy. There is reduction in each abdominal segment size in which the segment size in control larvae is 512µm, while it is 430 µm in the infected

larvae. The external body surface of the infected larvae was completely destructive and the main parts of the body could not be distinguished as in control **Figure (5 A, B).** The infection was strongly in thoracic legs and prolegs which reduce in size and appear short and stumpy with 212µm wide and 363 µm long while they 360 µm wide and 550 µm long in control larvae **Figure (5 C) .** Comparing the control larvae there is complete disappearance of crotchets (claws or hook like structure at the end of the prolegs help the attachment of larvae to the plants) as if they were pulled inside the larvae **Figure (5 D).** The infection is highly observed externally in the spiracles tissues component (responsible for filtrate the air for breathing) which is completely lacerated and profligate **Figure (5 E, F).** The spiracles in control larvae take oval shape with 77 µm wide and 139 µm long with healthy, organized and identity inside tissues with 49.6 µm wide and 116 µm long **Figure (4 E).** As a result of virus infection the oval shape spiracles converted to circular spiracles with 50.5 µm wide and 61.3 µm long with ruptured and disrupted inside tissue has 36.1 µm wide and 52.6 µm long **Figure (5 F) .**

There is exfoliation in some segments along the external body surface of the larvae specially segments contain damage spiracles **Figure (5 F).**

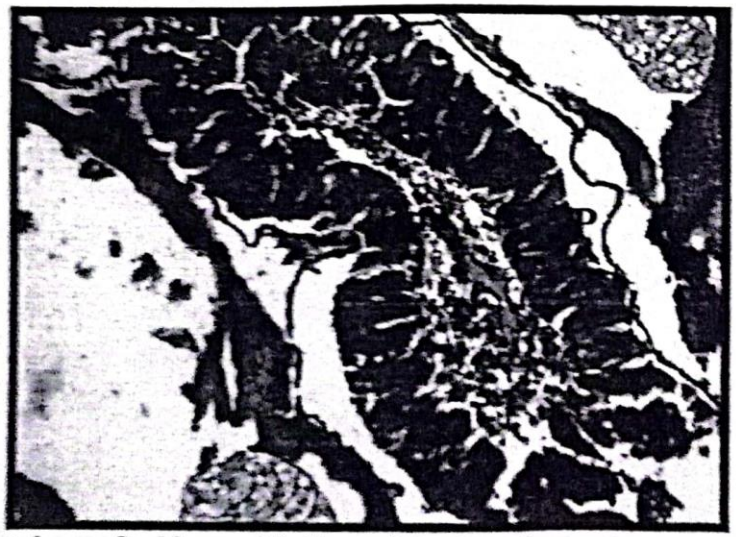

**Figure 1.** Histology of midgut epithelium of *S.exigua* larvae

**Pm** : Peritrophic membrane, **Bm** : Basement membrane,
**CC** : Columnar cells, **GC** : Goblet cells, **RC** : Regenerative cells.

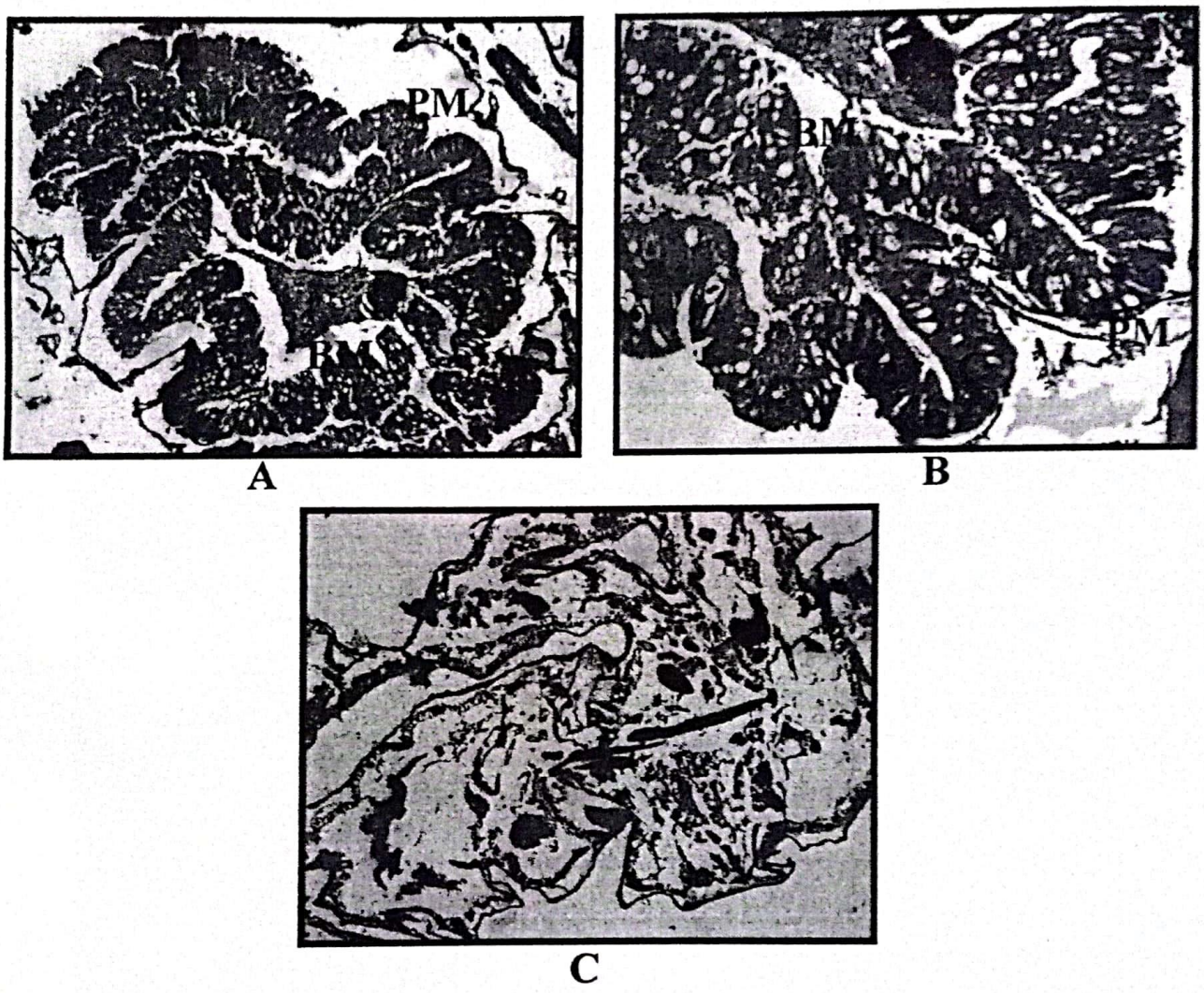


**Figure 2.** Histological sections of *SeNPV* midgut showing infection progression of *NPV* after different time of infection.

A and B: hyper-proliferation of the epithelial cells make the peritrophic membrane appear voluminous lead to formation of finger like projection also polyhedra and large number of small empty vacuoles scattered in different part of midgut.

C : complete destruction lead to liquefaction of all internal organs of the midgut.

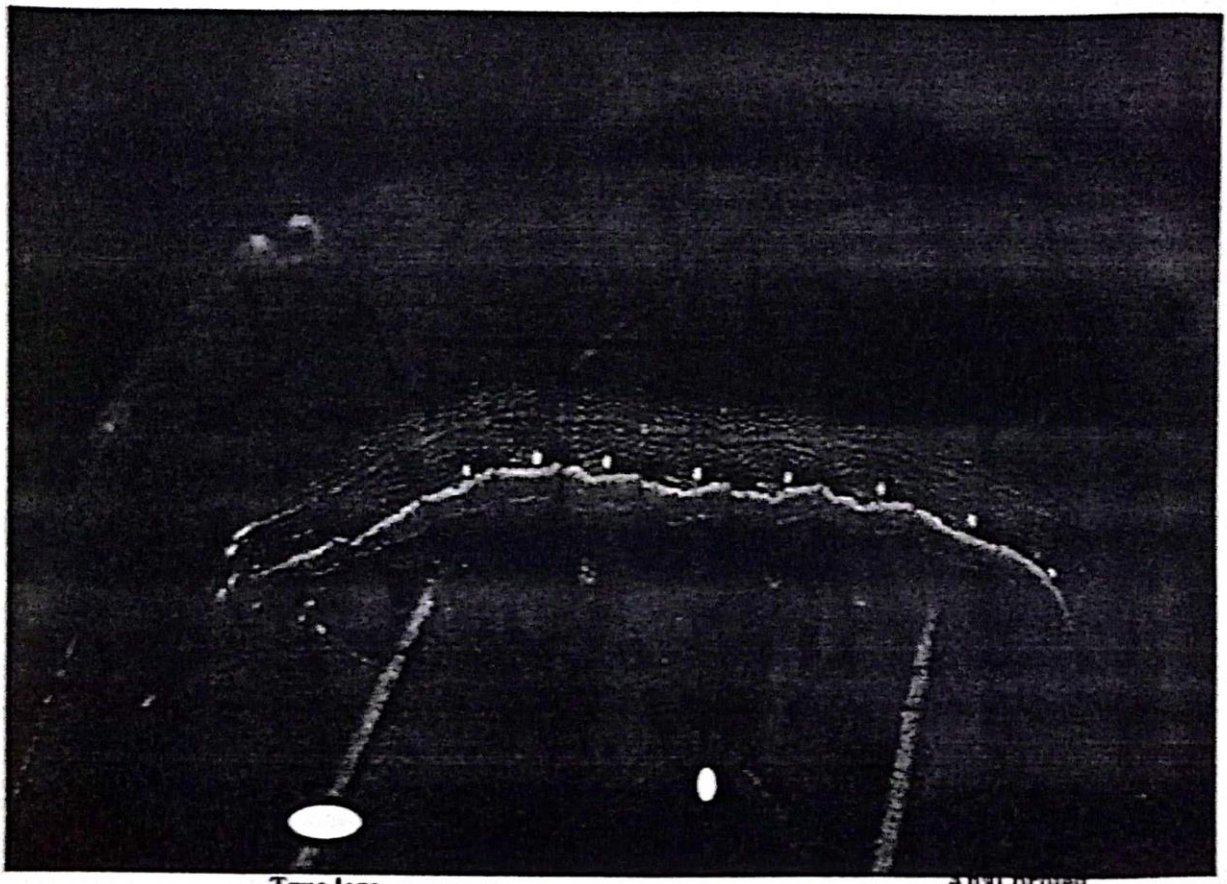


**Figure (3)** General structure of *S.exigua* larvae.

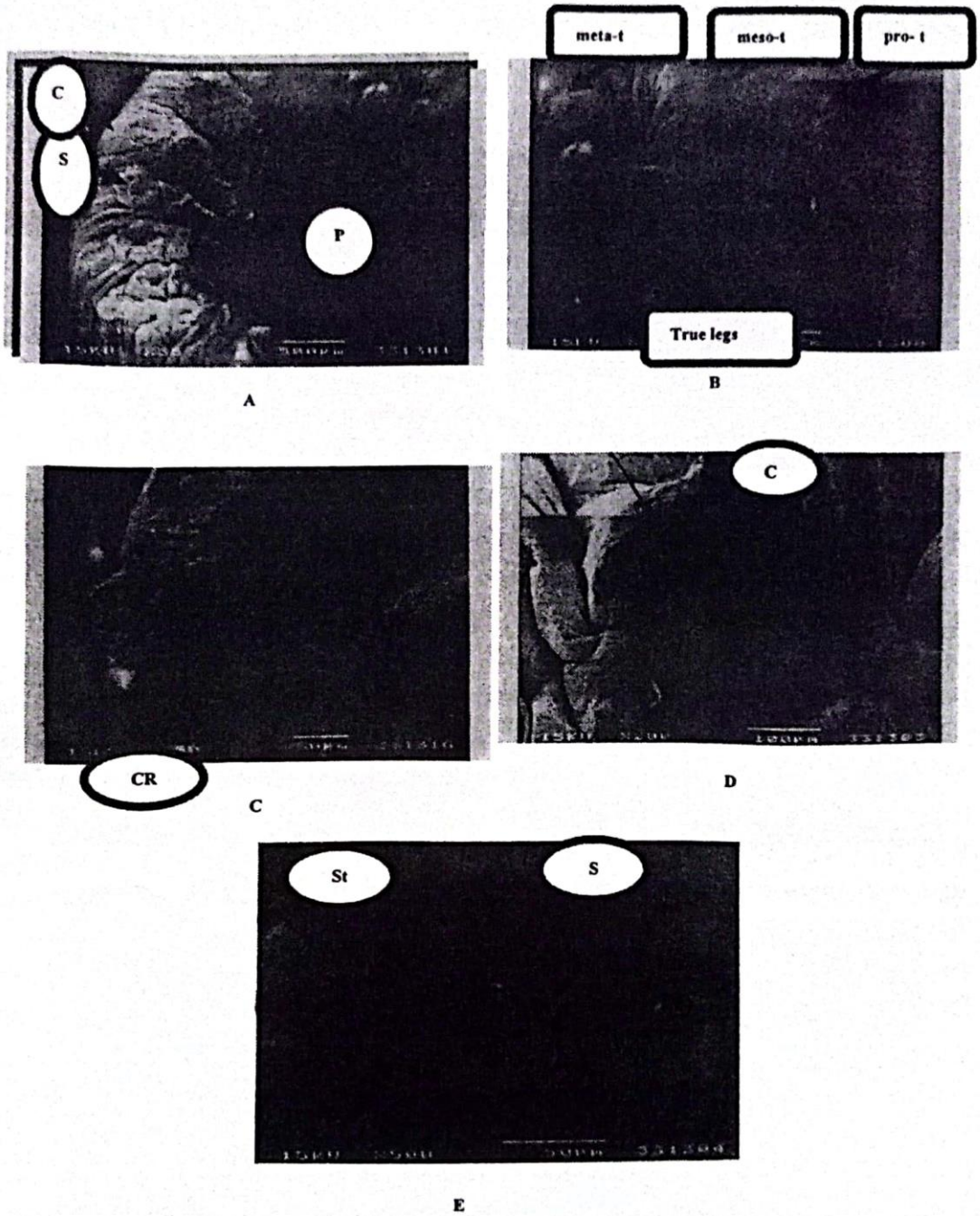


**Figure 4.** SEM micrograph of healthy *S.exigua* showing: A: abdominal segments reveals, intact cuticle (C), obvious spiracles (S), five pairs of prolegs (P). The abdominal segment size is about 512µm, B: division of thorax to three segments: pro-thorax (pro-t), meso-thorax (meso-t) and meta-thorax (meta-t), each segment bears a pair of legs (true legs). The size of thoracic legs is about 360µm wide and 550µm long, C: abdominal legs (prolegs) with visible crotchets (Cr), D and E: oval symmetric spiracle with about 77µm wide and 139µm long (S), organized spiracle inside tissue with about 94.6µm wide and 116 µm long (St), clear and intact cuticle surround spiracle (C).

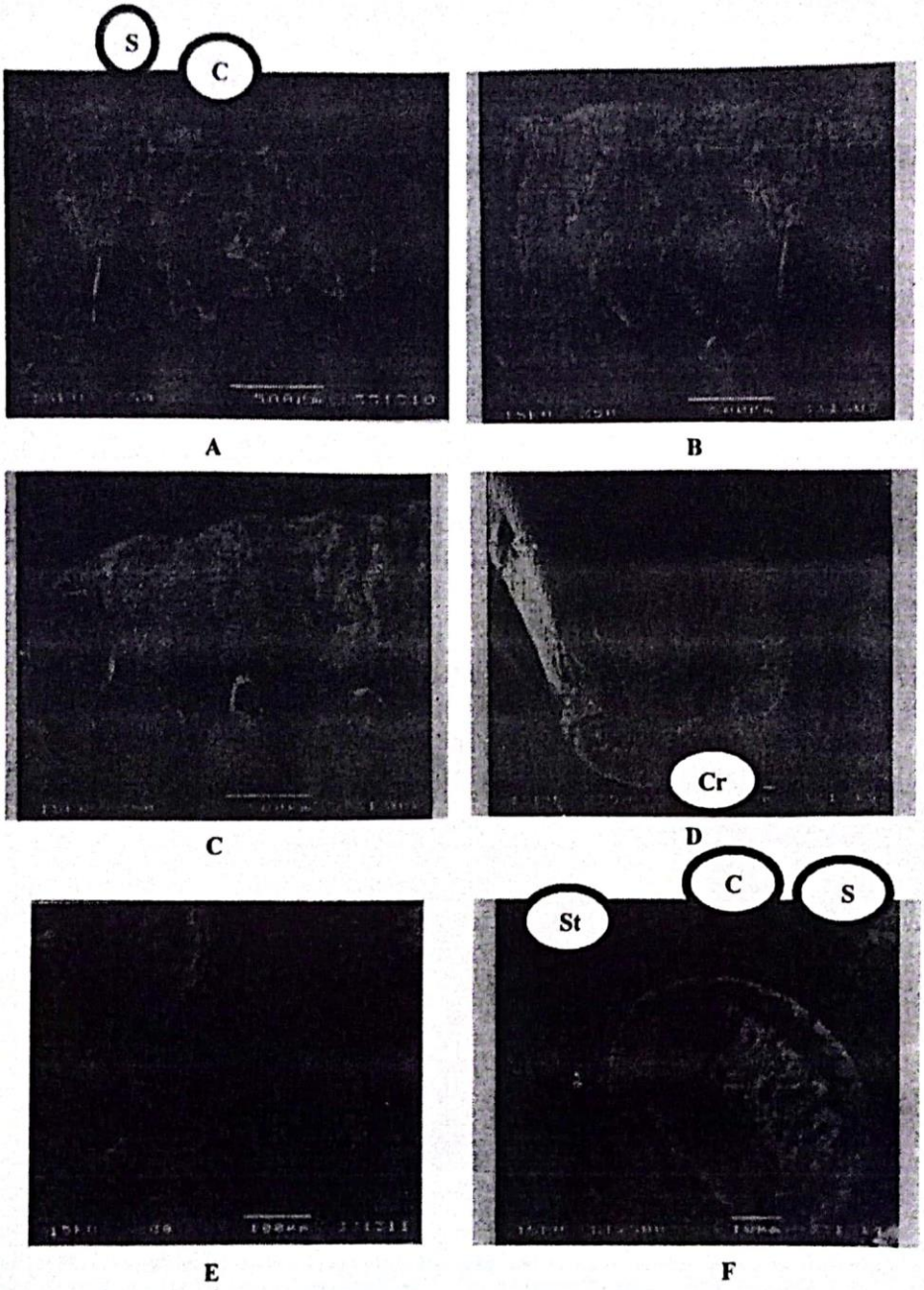


**Figure 5.** SEM micrograph of *SeNPV* showing: A and B: abdominal segments reflects, shrinking in all external body segments, the cuticle appear flaccid and completely damage (C), invisible spiracles as if buried inside shrinking cuticle (S). There is reduction in each abdominal segment size ranging from 250 to 430 µm, C: thorax segments with complete destruction of all external body surface and thoracic legs reduce in size and appear paralyzed with about 212µm wide and 363µm long, D: contracting of crotchets of prolegs, E and F: asymmetric spiracle with about 50.5µm wide and 61.3µm long (S), destructive and wasteful spiracle inside tissue with about 36.1µm wide and 52.6 µm long (St), exfoliated and granulated cuticle surround spiracle (C).

## DISCUSSION

In the present work damaging and abnormalities observed on the external larval body is the first evidence of the viral infection. Our histological and external morphological studies aimed to finding a possible relationship between the progression of infectivity and histological changes of the midgut as a result of feeding of *S.exigua* larvae on semi-synthetic diet treated with different concentrations of *SeNPV*.

To understand the *SeNPV* infection cycle, a brief overview of the *S.exigua* gastrointestinal tract is necessary, since this is the site of the initial infection and several major features of baculovirus biology have exploit this unique environment **Lehane and Billingsley (1996).** The *S.exigua* gastrointestinal tract is composed of three sections, the fore-, mid- and hindgut. The foregut is involved in facilitating the uptake, storage, and physical processing of food. It is lined with a chitin-containing cuticle that is part of the insect exoskeleton. The midgut is the major site of digestion of food and lacks a cuticle, but is lined with the peritrophic membrane (PM). The PM is composed of chitin, mucopolysaccharides, and proteins. It is thought that it protects the gut surface from damage caused by abrasive food material and to limit the access of microorganisms. It also allows the transfer of liquid and digested substances to the midgut epithelial cells, but prevents the passage of larger food particles. It is regenerated from the epithelial cells. The most common midgut epithelial cells are columnar cells with a brush border that is adjacent to the gut lumen. Regenerative cells are present at the base of the epithelium and they replenish the columnar epithelial cells that become damaged and are sloughed into the lumen. Goblet cells are also present and may be involved in ion transport that regulates pH. The midgut is involved in enzyme secretion and absorption of digested food and has a gradient of pH values. At the entry and exit of the midgut, the pH is near 7.0, but in the central region it can vary from 10.0 to as high as 12.0 **Dow (1992).** The hindgut is lined with a cuticle similar to the foregut and is involved in uptake of digested material. The general morphology of midgut epithelium in *S.exigua* larvae is similar to that described for many Lepidoptera such as *Manduca sexta* L. **Cioffi (1979),**

*Erynnis ello* L. **Santos *et al.* (1984),** *S.frugiperda* Smith **Jordao *et al.* (1999)** and *Anticarsia gemmatalis* Hubner **Levy *et al.* (2004).**

*SeNPV* infection starts when *S.exigua* larvae feed on semi-synthetic diet treated with different concentrations of *SeNPV*. The OBs dissolve in the alkaline medium of the larval midgut and liberated virions which enter into the epithelial cells, replicate in the nuclei thereby damaging the entire cellular texture of the midgut. Virions initiate the infection in the columnar epithelial cells that are a major cell type lining the midgut, and also regenerative cells of the larvae **Flipsen *et al.* (1995).** There are combinations of factors that appear to be involved in the initiation of midgut infections. These include factors that facilitate binding to the cells, cell receptors to which the virions bind, and virions envelope proteins that may have enzymatic activities that allow viral access to midgut cells or that fuse with the host cell membrane thereby permitting viral entry. A set of gene products called per os infectivity factors (pif) is required for infection of midgut cells. Four such genes have been identified including p74-pif (Ac138), Ac 22 (pif-2), Ac115 (pif-3), and Ac119 (pif-1). These four genes are conserved in all sequenced baculovirus genomes. This provides further evidence for a related pathway of infection for all members of this virus family. These genes have been implicated as ODV envelope-associated proteins **Fang *et al.* (2006)** and mediate specific binding of ODV to midgut cells, suggesting that they are directly involved in virus-cell interaction as an initial step in infection **Ohkawa *et al* 2005.** It has been suggested that ODV binds to proteinase sensitive receptors **Horton and Burand (1993)** then fuses with the epithelial cell membrane releasing the nucleocapsid into the cell cytoplasm.

*SeNPV* also encode endopeptidases (metalloproteinases) which considered integral components of their structure. One category of metalloproteinases, called enhancins, is concentrated in occlusion bodies. Enhancins greatly increased the permeability of PM by digestion of mucin and the PM component, thereby allowing virus access to the epithelial cell surface **Wang and Granados (1997).**

In addition to enhancins *NPV* chitin binding proteins play an

important role in damaging PM which composed mainly of chitin. The chitin component of the PM is produced by chitin synthase, an enzyme that is located at the apical tips of brush border microvilli. The enzyme is also found associated with tracheal cells **Broehan *et al.* (2007).** Therefore, an affinity for chitin could facilitate the interaction of the virion with these cells, first to initiate infection of midgut cells and subsequently to interact with tracheal cells and make complete destruction of spiracles. We should noted that the tracheal cells have projections that penetrate through the larval body **Maina (1989).** Such projections could provide access to the tracheal system and allow the virions spread the systemic infection **Engelhard *et al.* (1994).** Further evidence for the ability of trachea to spread infections systemically was the observation that infections could be initiated by exposure of insects to BV through their spiracles. Such infections spread throughout the insect following tracheal tracts **Kirkpatrick *et al.* (1994) .**

Egt enzyme (ecdysteroid UDP-glucosyltransferase). Another *NPV* protein that can affect the infection. Egt are found in all lepidopteran *NPV* genomes. It play a very important role in insect steroid metabolism. The function of the viral Egt is to block molting and pupation in infected larvae by catalyzing the transfer of glucose from UDP-glucose to ecdysteroids, thereby inactivating these insect molting hormones **O'Reilly and Miller (1990).** Egt also prolongs the feeding stage of infected larvae, thereby allowing the virus to replicate over a longer period of time in larger larvae, resulting in a higher yield of virus.

Disintegration of all organs of the midgut epithelial cells is also evident in the present work. Enzymes facilitating insect disintegration: Chitinase and cathepsin. Cathepsin cleaves key bone matrix proteins and is believed to play an important role in degrading the organic phase of bone during bone resorption. Cathepsin participate Chitinase in the liquefaction of whole larval body.

Another factor that facilitate disintegration is the high disturbance of morphology of fatty tissues which might a reason for cessation of feeding and death of infected larvae due to starvation,

since fatty tissues are the main organs of storage of nutrients and also for the supply of various constituents for the development of other tissues, so the principal sites of infection were the fat bodies and midgut. Similar results were reported by **Hamm, Styer and Lewis (1992).**

Development of shrunken body and vacuolization which was observed by SEM in our work may be explained here as the damaged midgut cells hinder the digestive process and damaged midgut wall, fatty tissues and connective tissues ultimately give rise to shrunken larval body, while the formation of vacuole was as a result of systematic infection and damaging of all internal larval organs. These results are in agreement with those of **Flipsen *et al.* (1995).**

## REFERENCES

**Addy, N. D (1969).** J. Econ. Entomol. 62, 270-271.

**Bancroft, J.D and Stevens, A. (1996).** Theory and practice of histological technique 4th ed, Churchill, Livingstone, New York, London, San Francisco, Tokyo.

**Blissard, G. W. and Rohrman, G. F. (1990).** Baculovirus diversity and molecular biology. ***Ann. Rev. Entomol., 35: 127-155.***

**Brochan G., Zimoch L., Wessels A., Ertas B. and Merzendorfer H. (2007).** A chymotrypsin-like serine protease interacts with the chitin synthase from the midgut of the tobacco hornworm. ***J Exp Biol. 210(Pt 20): 3636–43.***

**Cioffi, M. (1979).** The morphology and fine structure of the larval midgut of a moth (*Manduca sexta*) in relation to active ion transport. ***Tissue Cell 11: 467-479***

**Dow J.A. (1992).** pH gradients in lepidopteran midgut. ***J Exp Biol. 1992; 172(Pt 1): 355–375.***

**Engelhard E. K., Kam-Morgan L. N., Washburn J. O. and Volkman L.E (1994).** The insect tracheal system: a conduit for the systemic spread of *Autographa californica* nuclear polyhedrosis virus. ***Proc Natl Acad Sci U S A. 91: 3224–3227.***

**Fang M., Nie Y., Wang Q., Deng F., Wang R., Wang H., Wang H., Vlak J.M., Chen X. and**

**Hu Z. (2006).** Open reading frame 132 of *Helicoverpa armigera* nucleopolyhedrovirus encodes a functional per os infectivity factor (PIF-2). ***J Gen Virol. 87(Pt 9): 2563–9.***

**Flipsen, J. T. M., Mans, R. M. W., Kleefsman, A.W.F., Knebel-Morsdorf,D and Vlak, J. M. (1995).** Deletion of baculovirus ecdysteroid UDP-glucosyltransferase gene induces early degeneration of Malpighian tubules in infected insects. ***Journal of virology 69: 4529-4532.***

**Hamm, J. J., Styer, E. L. and Lewis, W. J. (1992).** Three viruses found in the braconid parasitoid Microplitis croceipes and their implications in biological control programs. ***Biol. Control. 2, 326-329.***

**Harley, M. M. and Fergusen, I. K. (1990).** The role of SEM in Pollen Morphology and Plant systemic. In: Scanning Electron Microscope Studies in Taxonomy and Functional Morphology. Ed by Clangher D, Systemics Association. Special Volume, 41 pp 45-68, Clarendon press. Oxford.

**Horton H. M. and Burand J. P. (1993).** Saturable attachment sites for polyhedron-derived baculovirus on insect cells and evidence for entry via direct membrane fusion. ***J. Virol.*** 67: ***1860–1868.***

**Hughes, P. R. and Wood, H. A. (1981).** A synchronous peroral technique for the bioassay of insect viruses. ***J. Invert. Pathol., 37, 154-159.***

**Jordão, B. P., Capella, A. N., Terra, W. R., Ribeiro, A. F. and Ferreira, C. (1999).** Nature of the anchors of membrane-bound aminopeptidase, amylase, trypsin a secretory mechanism in *Spodoptera frugiperda* (Lepidoptera) midgut cells. ***J. Insect Physiol. 45: 29-37.***

**Kikhno, I. S. Gutierrez, L. Croizier, G. Croizier and Ferber, M. L. (2002).** Characterization of pif, a gene required for the per os infectivity of *Spodoptera littoralis* nuclear polyhedrosis virus. ***J. Gen. Virol., 83: 3013-3022.***

**Kirkpatrick B. A., Washburn J. O., Engelhard E. K. and Volkman L. E. (1994).**

Primary infection of insect tracheae by *Autographa californica* nuclear polyhedrosis virus. ***Virology. 203(1): 184–6.***

**Lehane, M. J. and Billingsley, P. F. (1996).** Biology of the insect midgut. London, ***Chapman and Hall, 486p.***

**Levy, S. M., Falleiros, A. M. F., Gregório, E. A., Arrebola, N. R and Toledo, L. A. (2004).** The larval midgut of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae): Light and electron microscopy studies of the epithelial cells. ***Braz. J. Biol. 64: 1-8.***

**Maina, J. N. (1989).** Scanning and transmission electron microscopic study of the tracheal air sac system in a grasshopper Chrotogonus senegalensis (Kraus)--Orthoptera: Acrididae: Pyrgomorphinae. ***Anat Rec. 223(4): 393–405.***

**Mustafa Yaman, Remziye Nalcacioglu and Zihni Demirbag (2001).** Viral control of the European Pine Sawfly. *Neodiprion sertifer* (Geoffroy) in Turkey. ***Turk J. Biol. 25: 419-425.***

**Ohkawa T., Washburn J.O., Sitapara R., Sid E. and Volkman L.E (2005).** Specific binding of *Autographa californica* nucleopolyhedrovirus occlusion-derived virus to midgut cells of Heliothis virescens larvae is mediated by products of pif genes Ac119 and Ac022 but not by Ac115. ***J. Virol. 2005; 79: 15258–64.***

**O'Reilly D. R. and Miller L. K (1990).** Regulation of expression of a baculovirus ecdysteroid UDPglucosyltransferase gene. ***J. Virol. 64: 1321–1328.***

**Santos, C. D., Ribeiro, A. F., Ferreira, C and Terra, W.R (1984).** The larval midgut of the cassava hornworm (Erinnyis ello): Ultrastructure, fluid and fluxes and the secretory activity in relation to the organization of digestion. ***Cell Tissue Res. 237: 565-74.***

**Shorey, H. J., and Hale, R.L (1965).** Mass rearing of larvae of nine noctuid species on simple artificial media. ***J. Econ. Entomol. 58: 522-524.***

**Smits, P. H. (1987).** Nuclear polyhedrosis virus as biological

control agent of *Spodoptera exigua*. PhD. Thesis, Agric. Univ. Wageningen, the Netherlands, 127 p.

**Trumble, J. T., and Baker, T. C (1984).** Flight phenology and pheromone trapping at *Spodoptera exigua* (Hubner.) (Lepidoptera: Noctuidae) in southern coastal California. ***Environ. Entomol. 13: 1278-1282.***

**Volkman, L. E. and Keddie, B. A. (1990).** nuclear polyhedrosis virus pathogenesis. ***Seminars in Virology 1, 249-256.***

**Wang P., Granados R. R (1997).** An intestinal mucin is the target substrate for a baculovirus enhancin. Proc Natl Acad Sci U S A. ***94(13): 6977–82.***

**Yasmein, A, El Sayed, El-Dougdoug, K. A., Bekheet, H. K. and Ebrahim, A. G. (2010).** Isolation and Identification of *Spodoptera exigua nuclear polyhedrosis virus*. ***Egyptian Journal of Virology***, In Press.

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