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CYTOPATHIC AND HISTOLOGICAL EFFECTS OF INFECTED *PHTHORIMAEA OPERCULELLA* ZELLER (POTATO TUBER MOTH) WITH GRANULOSIS VIRUS (*POGV*)

[35]

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ABSTRACT

The potato tuber moth *Phthorimaea operculella* is a potato major pest in Egypt. Larvae of *P.operculella* were inoculated with a granulosis virus (*PoGV*) showed different cytopathic effects . *PoGV* induced characteristic symptoms of slow motion, white milky appearance and swilling of the body . Light micrograph of *PoGV* infected larvae figured out several histological changes. It was found that the midgut epithelial cells were the main 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 red colour appeared due to presence of granules particles . The morphological structures of *P.operculella* infected larvae with granulosis virus as well as healthy ones were examined by SEM . It showed complete destruction of external larval body , abdominal segments overlapped in some , prolegs and thoracic legs reduced in size and also the circular shaped spiracles converted to oval shape with rupture and disrupted inside tissue compared to healthy one.

Keywords : *Phthorimaea operculella* , granulosis virus , morphological , histological.

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INTRODUCTION

The potato tuber moth (PTM) *Phthorimaea operculella* Zeller (Lepidoptera, Gelechiidae) is a cosmopolation pest of potato causing significant losses in many tropical and subtropical regions **(Radcliffe, 1982 ; Cisneros and Gregory, 1994)** . larvae mine in both leaves and tubers , at the field and store . This feeding behaviour makes the pest difficult to control . Insect viruses are biological control agents having fewer detrimental effects on the environment, non target organisms and humans than chemical pesticides . Most viral agents used to control pests are either nucleopolyhedroviruses (NPVs) or granuloviruses (GVs), both of which belong to the Baculoviridae . The digestive tract of insects is considered an effective physical and chemical barrier against potentially invasive pathogens that are ingested with food **(Levy *et al.*, 2004)** The infection starts when the caterpillar present in treated tuber and ingests the granule that are dissolved and whose derived virions are released in the midgut lumen. These virions supposedly pass through the peritrophic membrane and fuse with the microvillar membrane of the midgut epithelial cells, initiating the replication cycle **(Flipsen *et al.*, 1995; Volkman, 1997)**. The present work was carried out to investigate the effect of *PoGV* on anatomical and scanning changes in *P.operculella* larvae by using SEM as well as histological structure of their midgut epithelial cells using light microscope.

MATERIALS & METHODS

Insect and virus source:

The used laboratory strain of PTM was reared under laboratory conditions away from insecticides for more than ten generations . The *PoGV* was provided by Insect Pathogen Production Unit of Plant Protection Research Center , Agricultural Research Center

Infection of tests larvae :

An Egyption isolate of *PoGV* was maintained on PTM larvae . One infected larva was homogenized in sterilized water and the suspension was adjusted to a final volume of 1 liter . The suspension was sprayed to contaminate small potato tubers . *P.operculella* eggs were laid on small pieces of filter paper and then placed on treated tubers . Newly hatched larvae fed on surfacely contaminated tubers with occlusion bodies. Larvae developed under

controlled conditions at 27 ^{0}C and relative humidity of 80%. Diseased larva instars were collected after 7 to 12 days to investigate the cytopathic effects on larvae .

Scanning electron microscopic examination (SEM) :

Healthy and infected larvae of PTM immediately fixed in glutaraldehyde (2.5 %) for 24 h period at 4^{0}C , then post – fixed in osmium tetraoxide (1 % OsO_4) for one hour at room temperature **(Harley and Fergusen, 1990).** Samples were dehydrated with bathing through ascending concentrations of acetone , the samples were dried till the critical point . Finally , larvae were sputter coated with gold . The examination and photographing were done through a JOEL Scanning Electron Microscope (T.330 A) in the Central Laboratory of Faculty of Agricultural , Ain Shams University .

Light microscopic examination :

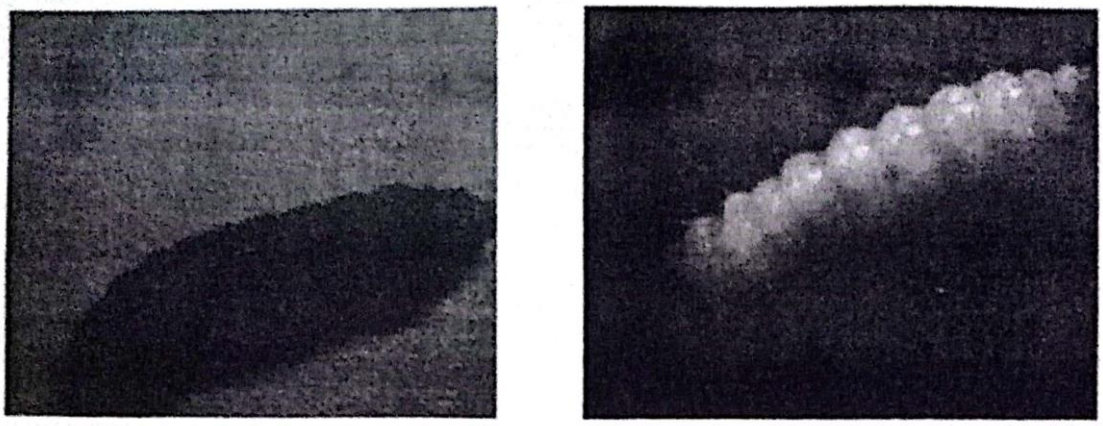
The infected and healthy larvae of PTM were dissected to small pieces and fixed in 10% neutral bufferd formalin , 1% glycerin and 1% thymol and Bouin ' solution . The samples were processed routinely by normal histological technique and sectioned at 4-5 µ thickness . the paraffin sections were stained with Haematoxylin and eosin according to **(Bancroft and Stevens , 1996).**

RESULTS

Healthy larvae of PTM has pinkish or greenish colour, wormlike and tubular bodies **(Figure 1a).**

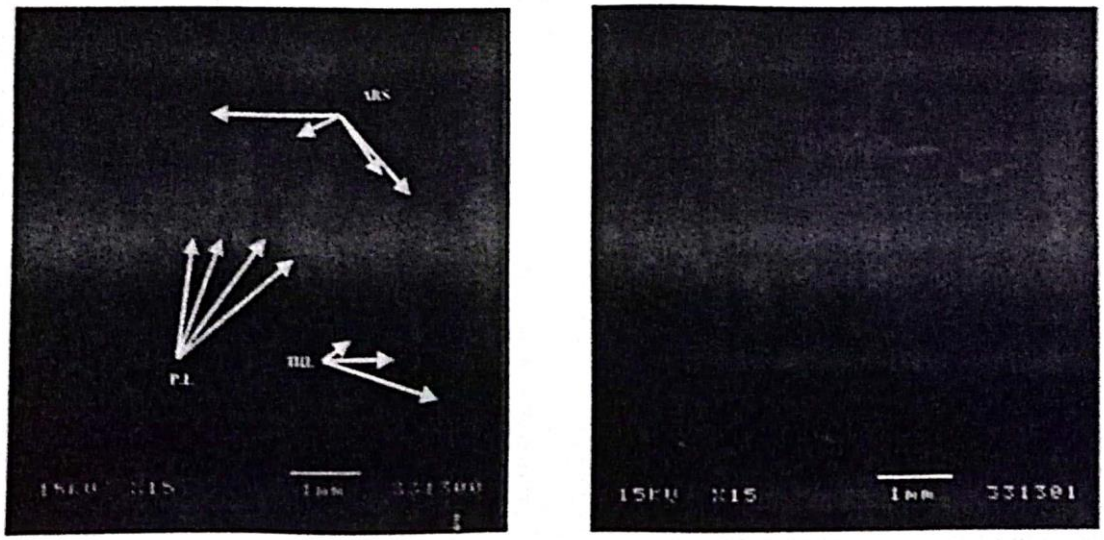
The first evidence to infection of the *P.operculella* larvae with GV is a change in its colour to milky white as in **Figure (1b).** The change in color is accompanied by progressive weakening, sluggishness and flaccidity of the larvae. In the late stage of infection, the integument of infected larvae ruptures and disintegrates generating a milky fluid. In addition, it became increasingly less responsive to stimuli. The mouth parts, mandibles and sensory apparati (eyes and antennae) showed abnormalities in shape and function. The scanning electron microscope monotoring the external changes of the larval buckle compared with healthy one **(Figure 2).** The body segments overlapped in some and with swollen segments **(Figure**

3). The movement of infected larvae showed paralysis and loss of ability to stick on its host wheather leaves or tubers .These changes become apparent in the form of thoracic ,abdominal legs and crotchets which exit from abdominal legs and can‹t enter again to complete its movement(**Figure 4,5 b**). Also, the spiracles of infected larvae lost its rounded normal form and converted into approximately oval shape and indulged in insect body (**Figure 6 b**). Death of infected larvae usually occurs during larval stage but sometimes it is delayed till the prepupal or pupal stages. Structure of midgut in healthy PTM larvae is shown in (**Figure 7a**). The histopathological studies of larvae treated with *PoGV* after 7 – 12 days of infection revealed the pathogenic effect of the virus on different tissues. The infection begins after ingestion of GV by newly hatched larvae. When granules 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 under light microscopy showed high degree of disintegration resemble in breakdown in the peritrophic, basement membrane, all the midgut cell (columnar, goblet and regenerative) lost their identity and an intensed red color throughout the cells appeared due to the presence of viral inclusions body **(Figure 7 b,c,d).**



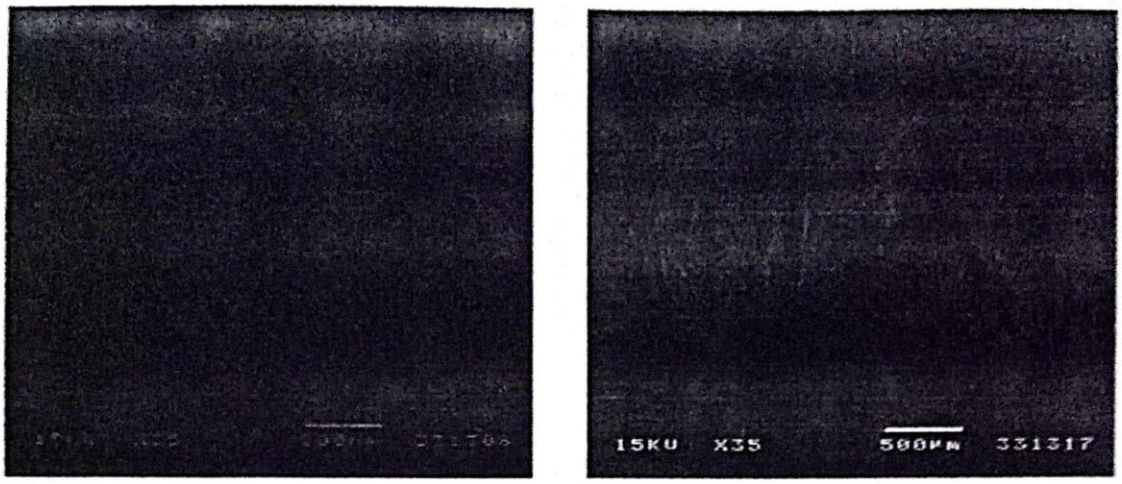
A B

Figure 1. larvae of *P.operculella* : (A) healthy with pinkish colour , (B) infected with GV changed into white milky colur and swollen.



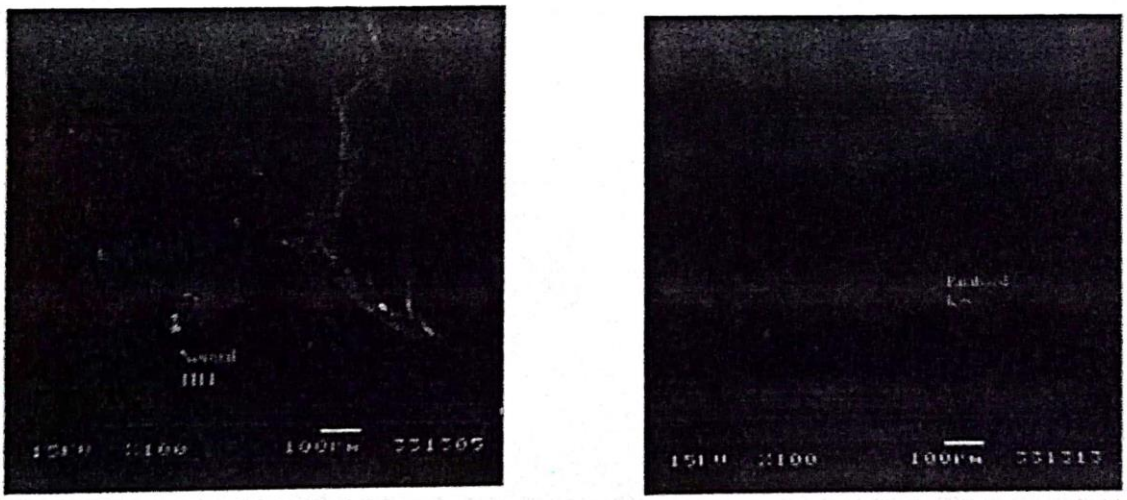
A B

Figure 2. SEM immage of *P.operculella* caterpillar: (A) healthy showing abdominal segments(AB.S), prolegs (P.L) , thoracic legs (TH.L) , (B) infected with granulovirus(*PoGV*) showing insect buckle.



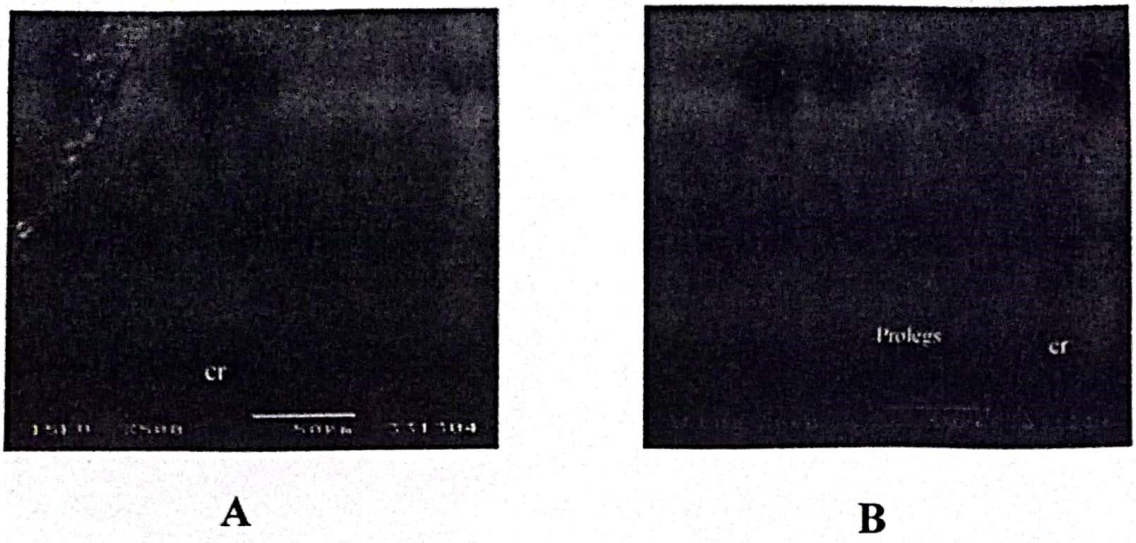
A B

Figure 3 . SEM immage of *P.operculella* abdominal segments (A) healthy segments , (B) segments of infected larvae swollen and overlapped in some



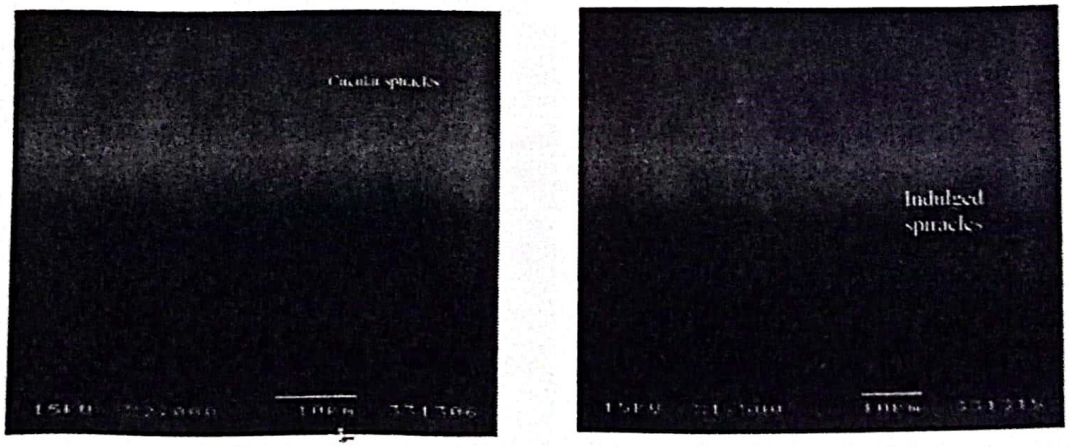
A B

Figure 4. SEM immage of *P.operculella* thoracic legs : (A) normal thoracic legs (TH.L) , (B) infected thoracic legs with paralyzed shape



A B

Figure 5. SEM immage of prolegs with crotchets (cr): (A) healthy and normal prolegs with crotchets (B) abnormalities prolegs in infected larvae



A B

Figure 6. SEM immage of *P.oprculella* spiracle: (A) healthy with circular and restricted shape , (B) infected oval shaped and indulged in larval body

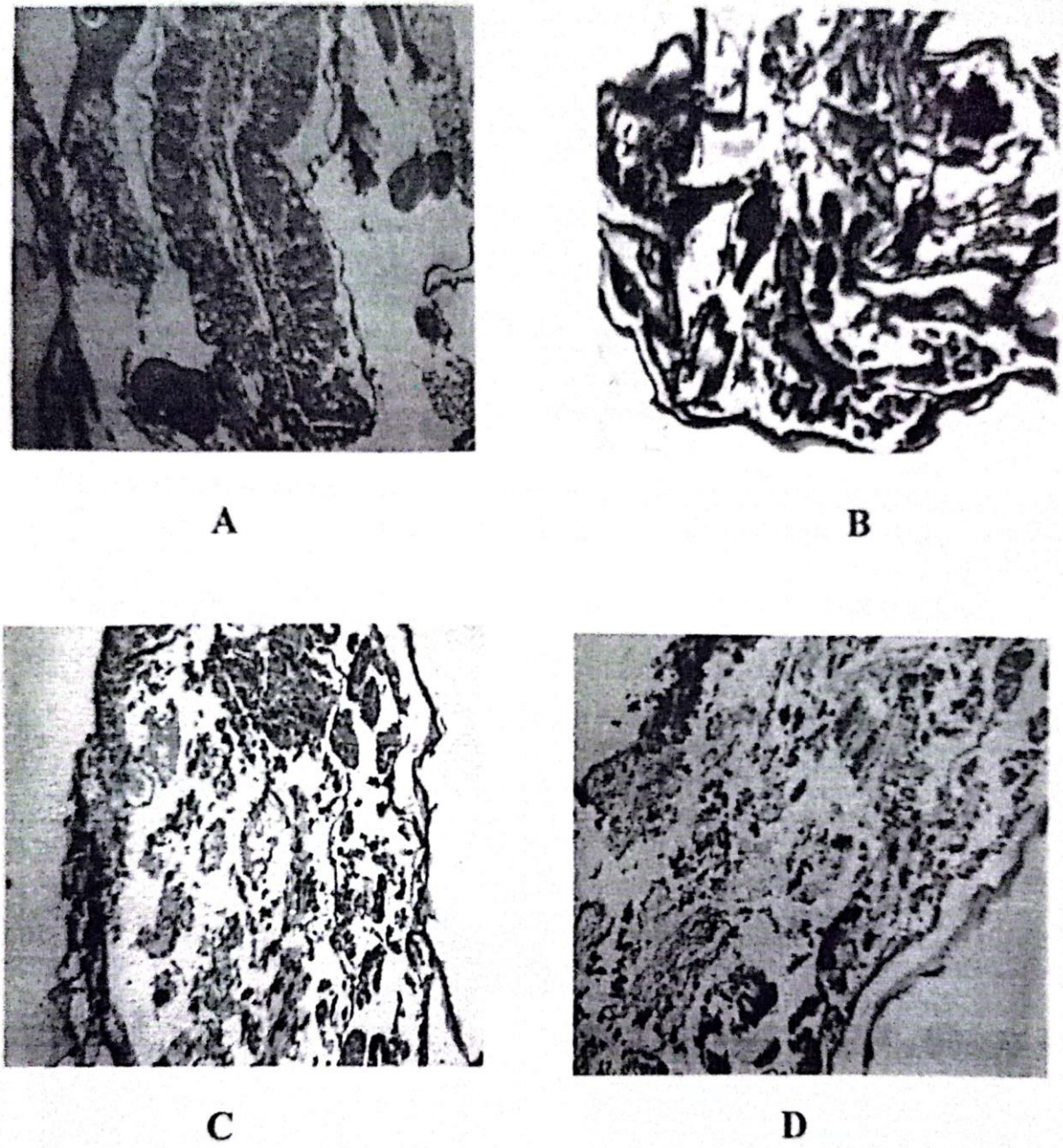


Figure 7. Light microscope image of epithelium midgut of *P. operculella* larvae showed : (A) healthy epithelium midgut composed of periotrophic membrane (PM) basement membrane (BM) regenerative cells (RC) ,columnar cells (CC) and goblet cells (GC) , (B) showed disintegration of cells and observed of red colour due to presence of GV, (C, D)showed complete destructive of midgut cells.

DISCUSSION

The internal changes in the midgut of *P.operculella* revealed to the external changes which observed on the larval body. In the larval digestive tract, the forgut and the hindgut are protected by a cuticle layer leaving the midgut as only region without a cuticle covering in the digestive tract actively interfacing with challenging environmental factors while performing the essential digestion and nutrient absorption functions. In lepidopteran larvae the peritrophic membrane (PM) is readily present at the time of larval hatching from eggs and immediately after ecdysis during the larval stages **(Wang and Granados, 1998)** . Lepidopteran larvae have Type 1 PMs which line the midgut epithelium and move posteriorly with the food bolus along the digestive canal **(Peters, 1992).**

The columnar cells, which have several names e.g: digestive cells, principal cells, absorptive cells ,and enterocytes, are predominant along the epithelium of the midgut wall of *P. operculella* larvae and show similar morphological aspects to other Lepidoptera species described by authors such as **(Cristofoletti *et al.*, 2001).**

The goblet cells described in the epithelial midgut of *P.operculella* larvae are similar to those found in other Lepidoptera species. They present a typical goblet-shaped cavity, called "goblet chamber". Into this cavity extend cell surface basal and lateral projections, similar to microvilli but filled with mitochondria. Apical projections form a valve type, which would permit the confluence of the cavity with the lumen, controlling fluid movements between. them **(Chapman, 1998).** According to **(Billingsley and Lehane , 1996)** the presence of mitochondria inside the projections is a unique characteristic in arthropods, is related to active transport of potassium ions from the hemolymph to midgut lumen, and calcium ions from adjacent columnar cells **(Koch and Moffett, 1995 ; Moffett *et al.*, 1995).** The presence of these cell types can explain the high pH (8.0-12.0) in the midgut of Lepidoptera and Ephemeroptera **(Terra, 1988).**

In our observation of different sections of infected larval tissues, *PoGV* infection was polyorganotropic and spread

throughout most tissues, For *GV*, three types of pathogenesis have been described based on tissue tropism in insect hosts (**Federici, 1997**) Type 1 is a typical granulosis in which the epidermis remains unaffected but other organs such as the fat body are infected. The virus invades the host via the midgut, and replicates mainly in the fat body. The virus slowly kills its host insect. Type 2 is a systemic *Granulovirus* in which most organs such as fat body, epidermis, tracheal matrix, muscle, nerve, malphigian tubules, and reproductive and glandular tissues are infected in a manner similar to that of the lepidopteran NPV, resulting in a fast killing of the insect. *PoGV* is consequently a type 2 GV. Type3 granulosis is characterized by tissue tropism only in the midgut epithelium and at present contains only one member ,*Harrissina brillans* GV (*HbGV*) (**Federici and Stern, 1990**).To explain *Baculovirus* movement within the insect body, it is generally accepted that the midgut epithelial cells are the first to be infected and support viral replication (**Hess and Falcon, 1987** and **Keddie *et al.*, 1989**) established that midgut infection occurred in both the columnar and regenerative cells of the midgut epithelium. The effect of *PoGV* on the development of pupal and adult stages of potato tuber moth is due to ecdysteroid UDP glucosyltransferase gene which produces an enzyme that conjugates ecdysteroids (insects molting hormones) with UDP-glucose or galactose (**Kelly *et al.*, 1995 and O'Reilly *et al.*, 1992**) . The next step in the disease cycle involves infection of other larval tissues. It has also been suggested that infected hemocytes play a significant role in the systemic infection, but the exact mechanism is not readily apparent (**Keddie *et al.*, 1989**) .

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