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Full Length Research Paper

Concomitant primary infection of the midgut epithelial cells and secondary infection of tracheal cells of *Helicoverpa armigera* by HA NPV (Nuclear Polyhedrosis Virus)

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Abstract

A study of histopathological changes due to the infection of *Helicoverpa armigera* Nuclear Polyhedrosis Virus (HANPV) in the third instar larvae of *H. armigera* at 24, 48, 72 and 96 hour interval was carried out in the laboratory. The histopathalogical sequential changes in the post infected *H. armigera* revealed the presence of infected area in the midgut epithelium at 72 hpi (hour post infection) suggesting that the midgut was the most common route of entry for the virus into an insect host. However, 96 hpi preparations showed virogenic tracheal epidermal cells suggesting that these cells were probably the secondary site of infection.

Keywords: Helicoverpa armigera, Nuclaer Polyhedrosis Virus, histopathology

Introduction

Helicoverpa armigera (Hub.), the gram pod borer, is a serious polyphagous pest of several cultivated crops and has attained global importance as an alarming pest (Cunningham, 1995). Widespread development of resistance to chemical insecticides, including the widely used pyrethroids has been reported in *H. armigera* (Reddy *et al*., 1991 and Kranthi *et al*., 2001). The problem of resistance to chemical has so worsened that it resulted significant crop losses due to the pests in India in 1996-97 recording 158 million US$ (Russell,1999) and causing widespread hardship especially amongst poor farmers. In addition to development of resistance in pest, indiscriminate and injudicious use of pesticides has grossly poisoned almost each component of the biospheres, caused resurgence of pests and reduction of natural enemies in agro ecosystem allowing rapid rebound of target pests and minor pests (Verma and Singh, 2000). Now a day's integrated approach is the noble idea to manage crop pests as it involves minimum load of pesticides in nature (Ahmad and Chandel, 2004 and Patel *et al*., 2009). Thus considerable emphasis is being laid on the use of nuclear polyhedrosis virus (NPV) as a microbial pesticide (biopesticide), which is species specific, ecologically safe and under certain conditions it causes epizootics in the field which provides added control of pests in nature (Caballero *et al*., 1992,Herz *et al*.,2003 and Shapiro-Iian *et al*., 2012). Although many authors have investigated the hitopathalogical changes with light and electron microscopy (Engelhard *et al*., 1994; Flipsen *et al*., 1995; Washburn *et al*., 1995 and Levy *et al*., 2009), but there are certain lacunae which need clear understanding about the primary, secondary site of infection and the route of entry of NPV in the host. Keeping this in view, the present work is aimed to investigate the histopathalogical sequential changes in the post infected dated gram pod borer.

Materials and Methods

Nuclear Polyhedrosis Virus (NPV)

The naturally NPV infected larvae of *H. armigera* were collected from the fields of local farmers at Jaipur (Raj), India and the virus has partially purified and propagated according to the procedure described by Ramakrishnan and Choudhari (1979). The number of polyhedral inclusion bodies (PIB) in the stock suspension was calculated

through improved Neubauer Haemocytometer CR Counting Chamber.

Test Insect

The culture of *H. armigera* was maintained in laboratory at 27±1°C temperature and 70±5% relative humidity (RH). Since larval growth was not uniform on natural diet, the test insects were reared on artificial diet according to Nagarkatti and Prakash (1974).

Histological Techniques

The dated third instar larvae of *H. armigera* were fed on artificial diet preimpregnated with 1×10^9 PIB/ml of virus suspension. Three replicates with ten larvae were exposed singly on the impregnated diet and allowed to feed for 24 hr., thereafter; the larvae were transferred on fresh diet by soft brush. In control, the larvae were provided virus free fresh diet. Normal and infected larvae were dissected at 24, 48, 72 and 96 hr respectively, after exposure, in normal saline solution (Davenport and Wright, 1985). The midgut was removed and cut transversely into small pieces and fixed overnight in a Bouin-Duboscq fixative. The fixed tissues were processed routinely for sectioning. Paraffin (M.P-60°C) was used to embed the tissue which was sectioned by microtome at 6µm and stained with haematoxylin and eosin. The stained section of tracheae and midgut of normal and infected larvae were examined under high power microscope (Nikon Opticphot) as well as oil emersion.

Results

An examination of the histological preparations of control larvae revealed that the midgut consisted of a single layer of epithelial cells lining a spacious lumen and invested by an outer coat of muscle layers which are disposed off in longitudinal and circular fashion, the longitudinal being the outer (Fig.1). Between the gut contents and epithelial cells, there is a thick microfibrillar meshwork i.e. peritrophic membrane. The epithelial cells of the midgut area are of columnar type resting on a thin membrane. Besides, on the luminal side some pear shaped buds of various sizes are associated with the normal columnar cells. Interspersed between the columnar cells are the groups of small cells, the replicating or regenerative cells. The goblet cells are also found interspersed between the columnar cells having a large flask shaped cavity. Numerous tracheae and tracheoles are also found associated with the midgut in the histological section.

Polyhedral Inclusion Bodies were invariably seen in the lumen of the midgut at 24 hr. post infection (hpi.) preparations (Fig. 2). These PIB's were not visible in lumen of midgut in 48 hpi preparations (Fig. 3). No morphological abnormality could be observed in them and cell types were morphologically very close to the cells observed in control preparations. However, in 72 hpi preparations, two morphologically different areas were observed: (i) The infected region having hypertrophied cells with disintegrated nucleus and vacuolated cytoplasm along with lysis of cell wall and numerous cells being discarded to the midgut lumen by cell sloughing (Fig. 4); and (ii) In few areas the columnar cells appeared as that in control, thereby giving appearance of non-infected area. In 96 hpi preparations similar histopathological changes were observed in the tracheal epidermal cells, but these cells appeared virus free in 72 hpi preparations (Fig. 5).

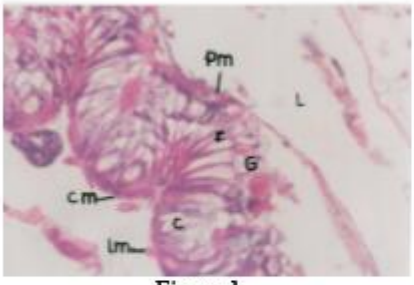

Figure 1

Light micrograph of midgut (cross section) of *H. armigera* (non- infected) showing lumen (L) and epithelium with columnar cells (C), goblet cells (G), regenerative cells (r), longitudinal muscle (lm), circular muscle (cm) and peritrophic membrane (pm), Ca×200; Eosin and haematoxylin.

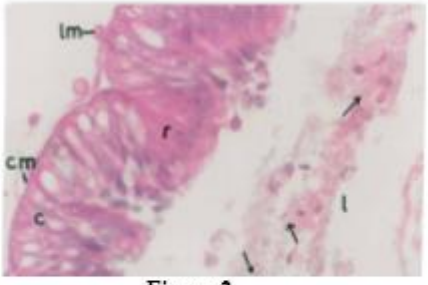

Figure 2

Light micrograph of midgut of H. armigera (cross section) of *H. armigera* infected with NPV at 24 hr.p.i showing PIBs (arrow ↑) in the lumen (L) and normal epithelium as in non-infected one, with columnar cells (C), regenerative cells (r), longitudinal muscle (lm) and circular muscle (cm), Ca×200; Eosin and haematoxylin.

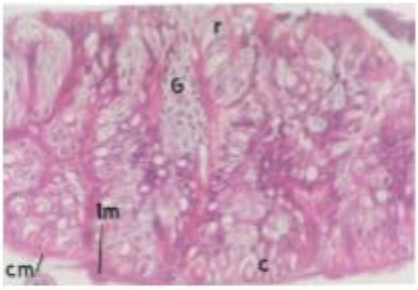

Figure 3

Light micrograph of midgut (cross section) of *H. armigera* infected with NPV at 48 hr.p.i showing non – infected columnar cells (C), goblet cells (G), regenerative cells (r), circular muscle (cm) and longitudinal muscle (lm), Ca×200; Eosin and haematoxylin.

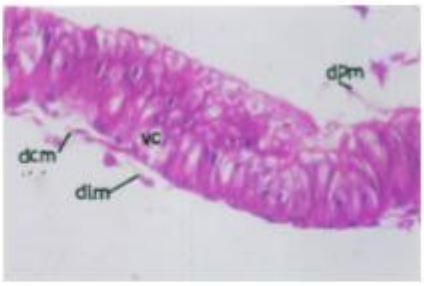

Figure 4

Light micrograph of midgut (cross section) of *H. armigera* infected with NPV at 72 hr.p.i showing detached longitudinal muscle (dlm) and circular muscle (dcm), vacuolated cytoplasm (vc) and disintegrated peritrophic membrane (dpm), Ca×200; Eosin and haematoxylin.

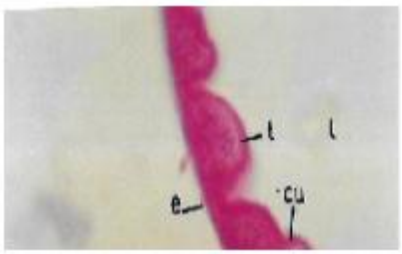

Figure 5a

Light micrograph of trachea (cross section) of *H. armigera* (non-infected) showing epithelium (e), taenidi(t), cuticle (cu) and lumen (l), Ca×200; Eosin and haematoxylin.

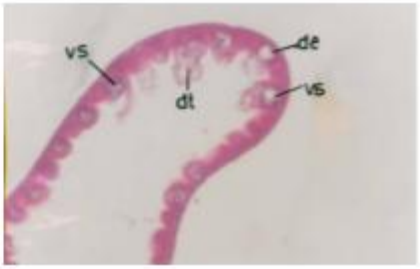

Figure 5b

Light micrograph of trachea (cross section) of *H. armigera* infected with NPV at 96 hr.p.i showing disintegrated epithelium (de) and taenidia (dt) with virogenic stroma (vs) and vacuoles (v), Ca×200; Eosin and haematoxylin.

Discussion

PIBs were only observed in the lumen of the gut at 24 hpi and thereafter no PIBs were visible in the lumen, their absence indicated that the occluding bodies were solubilized releasing the virion (which were not visible under the light microscopic resolution) by the action of highly alkaline intra-lumen environment (Granados, 1980; Volkman, 1997). These virions further fuse to microvilli and pass through the cytoplasm to nuclei (Hoston and Burand, 1993). Moreover, no morphological changes were observed in midgut epithelium at 48 hpi preparations which is in conformation with Matos *et al*. (1999) who found no virus or morphological changes in the first hour of infection in midgut epithelium of *Anticarsia gemmatalis*. However, in 72 hpi preparations, two distinct areas, the infected and non-infected areas were visible. The presence of non-infected area could possibly be due to the fact that midgut

epithelium is able to clear the infection after the first round of progeny virus production by cell sloughing. Such a view finds support from other workers (Washburn *et al*., 1995; Matos *et al*., 1999). In infected area, columnar cells were hypertrophied which is in agreement to the observations of other workers in midgut of *Anticarsia gemmatalis* (Matos *et al*., 1999), *Spodoptera litura* (Im *et al*., 1988), *Autographa californica* (Keddie *et al*., 1989), *Lymantria dispar* (Adams *et al*., 1994), *Spodoptera exigua* (Flipsen *et al.,* 1995; Knebel Morsdorf *et al*., 1995), *Agrotis segetum* (Oballe *et al*., 1996) and *Zethenia rufescentaria* (Lin *et al*., 1999). Therefore the midgut epithelium appears to play a role in providing the primary site for virus attachment and route of entry into an insect host (Flipsen *et al*., 1995; Washburn *et al*., 1996; Hoston and Burand, 1993; Knebel Morsdorf *et al*., 1996; Barett *et al*., 1998b). In 96 hpi preparations tracheal epidermal cells also showed marked histopathological changes, but not up to 72 hpi preparations; it appears probably that these cells are secondary site of infection (Matos *et al*., 1999; Barett *et al*., 1998a & 1998b) and subsequent dissemination of virus infection in the host is brought out by tracheal system (Engelhard *et al*., 1994; Adams and Bomani., 1991).

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