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Pathogenic virus and insect tissues: an effective way of pest control

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When different doses, i.e. 0.1, 0.25 0.5 and 0.75 ml of 2×10^9 polyhedra per ml of SlNPV were used against fourth instar larvae of *Spodoptera litura* (Fab) and observations were taken after 24, 48 and 96 h, *F*-value in CRD ANOVA was highly significant for doses as well as time period. Per cent mortality increased from 5.43 to 78.91 as the dose was increased from 0.1 to 0.75 ml of 2×10^9 polyhedra per ml solution. Various viral pathological symptoms such as flaccid and swollen body, stumpy legs, shrinkage of body segments at midgut area and extremely moist droppings were observed. Histopathological studies of fourth instar treated with highest dose, i.e. 0.75 ml, revealed the effect of the virus on various midgut cells, fat bodies, connective tissues and also on the integument. These tissues either lost their identity or became highly disorganized. The virions were found scattered in different tissues and deposited heavily on the body wall, suggesting the spread of infection in almost all the tissues of the larvae.

Keywords: Histopathological studies, pathogenic effect, *Spodoptera litura*, virions.

OVER reliance on pesticides and their indiscriminate use have resulted in insect resistance, resurgence, outbreak of secondary pests and residual toxicity[1]. With the advent of biotechnology and the beginning of ecologically friendly policies for pest control, microbial pesticides have achieved immense importance in plant protection programmes. Comparison of microbial pesticides with that of chemical pesticides is important from the perspective of their efficacy and cost. In addition to these benefits, microbial agents are safe for humans and other non-target species, are free from residual toxicity, preserve natural enemies and increase biodiversity of the ecosystem[2].

Microbial control as an important tool of Integrated Pest Management (IPM) uses entomopathogenic organisms,

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including bacteria, viruses, protozoans and nematodes[3]. Like other natural enemies, these pathogens can exert considerable control on target population and are responsible for decrease in insect population[4,5].

A large number of Baculoviruses offer potential as microbial control agents of insects. Amongst all the viruses, the greatest microbial potential is displayed by the Baculoviridae Nuclear Polyhedrosis Virus (NPV) and Granulo Virus (GV)[6]. More than four hundred insect species mainly from Lepidoptera and Hymnoptera have been reported as hosts of Baculovirus[7,8]. The efficacy, specificity and production of secondary inoculums make baculoviruses an attractive alternative to broad-spectrum insecticides and ideal components of the IPM system[9].

Spodoptera litura (Fabricius) (Noctuidae: Lepidoptera), commonly known as army worm or tobacco caterpillar, is an extremely serious pest, the larvae of which can defoliate many economically important crops. In Rajasthan, cole crops, cotton, groundnut and soybean crops are heavily damaged by *S. litura* (Fabricius). Methamyl and Rocket (Cypermethrin) are synthetic pesticides generally used against this pest. Heavy doses of pesticides cause pest resistance, resurgence and heavy contamination of the food chain. However, they are unable to check the pest population.

In the present study, microbial control of the pest has been tried using Spodoptera Polyhedrosis Virus (SlNPV) under the trade name Spodlure, against the fourth instar larvae of the pest (since these larvae are the most damaging stage of the pest). The efficacy of the viral biopesticide was adjudged by observing per cent mortality and abnormalities at different applied doses. These results were supplemented by studying the mode of action of the virus on various tissues of the insect. The complete study has thrown light on the use of the virus for control of the pest. The study will also supplement in devising different strategies for pest control.

Spodlure was procured from the Department of Entomology, Maharana Pratap Agricultural University, Udaipur. The viral preparation contained 2×10^9 polyhedra per ml. Considering this as stock solution, four different dilutions (viz. 0.1, 0.25, 0.5 and 0.75 ml) were prepared and fed to the fourth instar larvae of *S. litura* (Fab) by leaf-dip method[10]. Castor leaf of 6 cm diameter was dipped in prepared concentrations for 1 min and shade-dried for 30 min. Then leaf discs were placed in a slanting position in separate containers so that the larvae can feed on both the surfaces of the leaf. Ten fourth instar larvae were released in each container with three replicas and one control. Observations were made at 24, 48 and 96 h by taking per cent motality and morphological abnormalities into account. To supplement these results, histopathological sections were cut after 48 h of treatment at 0.75 ml of dose. For histopathology, whole body sections were taken passing through the midgut region. Tissues were fixed in Gilson fluid, washed in Lugol's iodine and 6 μm thick sections were cut and stained in Mallory triple stain.

Different doses of the virus brought about significant mortality, which was both dose- and time-dependent. At maximum period of treatment, i.e. 96 h mortality increased from 5.43 to 78.91%, from the lowest dose (0.1 ml) to the highest applied dose of 0.75 ml. It was highly significant compared to only 1% in control. The CRD ANOVA test was significant at 1% level. The *F*-value was significant for the treatment period also. Toxic nature of viral pesticide against many Lepidopteran pests has been also reported[11] Apart from mortality, various morphological abnormalities were also observed. These included shrinkage of body segments, stumpy legs, flaccid body and extremely moist droppings at later stages[12].

Histopathological sections clearly revealed the reasons behind the above-mentioned observations in virus-treated larvae. The histoarchitecture of controlled tissue revealed an uninterrupted outer cuticle divided into exo and endo cuticle. This layer is followed by the epidermal layer, compact fat bodies and midgut in the body cavity (Figure 1 *a*, *b*). Midgut is made up of epithelium consisting of columnar epithelial cells, goblet cells and regenerative cells in the crypts. These cells are separated from the lumen by continuous peritrophic membrane[12] (Figure 1 *c*).

In the whole body section of virus-treated tissues, architectural dystrophy was observed in almost all tissues of the larval bodies. Since the virus begins to act only after being ingested through the food and getting activated in the alkaline pH of the midgut[13], this was the first organ targetted by the virus. The entire midgut area along with the muscular layers and basement membrane were found shrunken in the body cavity compared to control, where different cells of the midgut are clearly distinguished (Figure 1 *c*). In the treated section, midgut was folded in a characteristic manner and the inner part was occupied by large vacuoles (Figure 2 *a*, *b*).

Typical viral pathology was observed in fat bodies and connective tissues of the body cavity. The large, tightly packed fat cells with large nuclei in control (Figure 2 *c*) lose their identity in treated tissues. The fat cells were found swollen with atrophied nuclei heavily loaded with virions and large vacuoles. The parietal and visceral fatty layers could not be distinguished due to loss of connective tissues (Figure 2 *a*, *b*).

The body wall of the larvae was completely damaged. Endocuticle could not be distinguished from exocuticle as in control (Figure 1 *b*). Contents of endocuticle reduced significantly giving a faint blue colour to Mallory triple stain. It also became detached from the exocuticle forming space in between. The polyhedra were seen deposited in various crypts of the body wall (Figure 2 *d*, *e*)

The histomicrograph clearly 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 gut epithelial cells were the first targets of virus action. The entire midgut area along with the basement membrane

and muscular layers revealed complete dystrophication (Figure 2 *a*). The lumen of the gut was almost obliterated. Due to shrunken midgut, with tissues squeezed out from inside the gut, large empty vacuoles in the midgut area were also distinct (Figure 2 *b*).

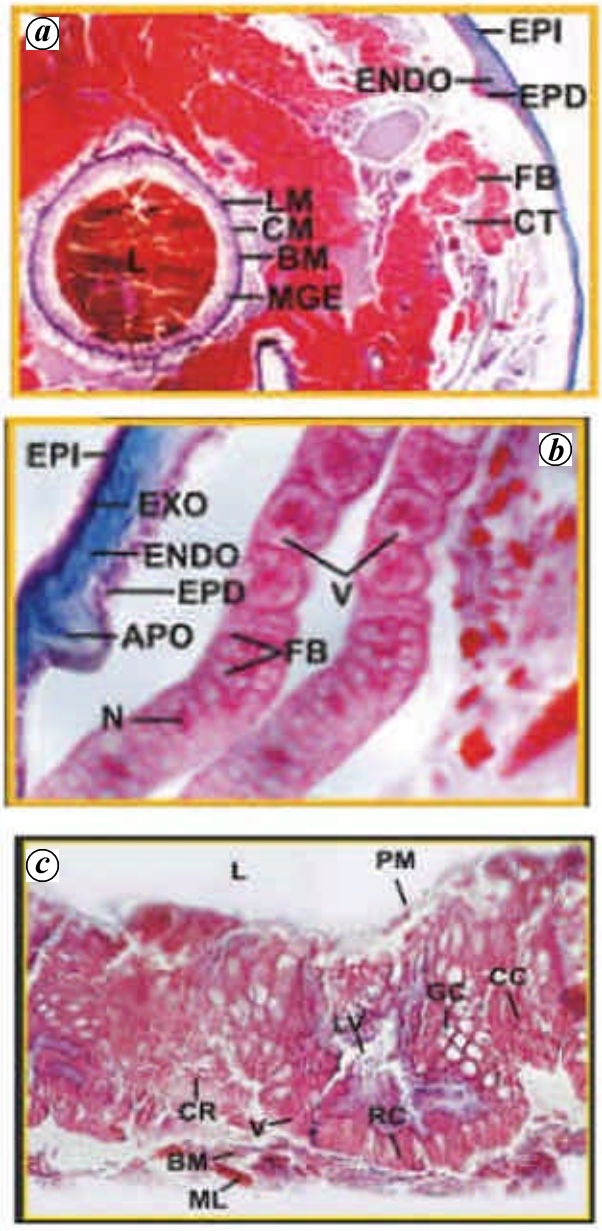

Figure 1. ***a***, TS of whole body section passing through midgut region of fourth instar larva showing following layers from outside to inside. EPI, Epicuticle; ENDO, Endocuticle; EPD, Epidermis; FB, Fat bodies; CT, Connective tissues; LM, Longitudinal muscles; CM, Circular muscles; BM, Basement membrane; MGE, Midgut epithelium, and L, Lumen; ×10. ***b***, Magnified view of part of cuticle along with fat bodies of fourth instar larva. Epicuticle (EPI), Exocuticle (EXO), Endocuticle (ENDO) and Epidermis (EPD) are well distinguished. Internal extension of epicuticle in the form of Apodeme (APO) is also significant. Fat body cells (FB) are clearly marked with vacuoles (V) and nucleus (N); ×100. ***c***, Magnified view of one complete crypt (CR) and villi (V) with large distinct goblet cells (GC), regenerative cells (RC), columnar epithelial cells (CC), peritrophic membrane (PM), muscular layer (ML), basement membrane (BM), lumen of villi (LV) and lumen (L) of midgut; ×40.

Virions replicating in the midgut epithelial cells attack on the integrity of the midgut tissues as a result of which they lose their identity and appear as a clumpy mass of debris. Our results are also in accordance with those of other authors who have reported that the midgut epithelial cells are severely affected by the virus[14,15]. Chitihunsa and Sikorowski[16] reported high concentration of Mc NOBV in most tissues of infected wasps, including the alimentary canal, fat body, circulatory system and the integument. In *Heliothis virescens*, the virus was present in the midgut, tracheal matrix, fat body and haemolymph[17]. After ingestion by the host, the Polyhedra are dissolved in the alkaline medium of midgut lumen and liberated virions enter into the epithelial cells, replicate in the nuclei thereby damaging the entire cellular texture of the midgut. Development of shrunken body and moist droppings 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. After infecting the midgut tissues, the budded virus particles enter the haemocoel through which the virus invades various tissues like fat bodies, trachea, connective tissues and integument. The fatty tissues of infected caterpillar display a strong phenotype with condensed regions of darkly stained tissues and presence of cavities in the normal contiguous fat bodies[18].

Dystrophied nature of fat bodies with swollen cells, and large and coarse nuclei at the verge of disintegration are also evident in the present study. Highly disturbed morphology of fatty tissues might be 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. *Spodoptera frugiperda* infected with Ac MNPV revealed that although haemocytes played an important role in spreading infection, infection of fat bodies and epithelial cells was observed prior to the infection of haemocytes[19]. The principal sites of infection in *Microplitis croceipis* wasps were the fat bodies and midgut[20]. Infection in the fat body occurs in close proximity to the trachea, and infection was strong in the thoracic legs, prolegs and mid-region of each abdominal segment. Baculoviruses are able to cross the midgut basal lamina before the midgut epithelium is sloughed-off and enter into other organs and spread infection rapidly deep into the tissues of their hosts[21,22]. Engelhard *et al.*[22] reported the spread of infection from midgut across the basal lamina and into the body of the caterpillar via tracheoblast cells. Once the virus enters the body of the caterpillar, infection occurs simultaneously within the haemocoel in the tracheal system of the host insect and spreads rapidly to the epithelial and fat body tissues.

The cuticular layers are also damaged severely. Due to disturbances in chitin deposition, the integument became

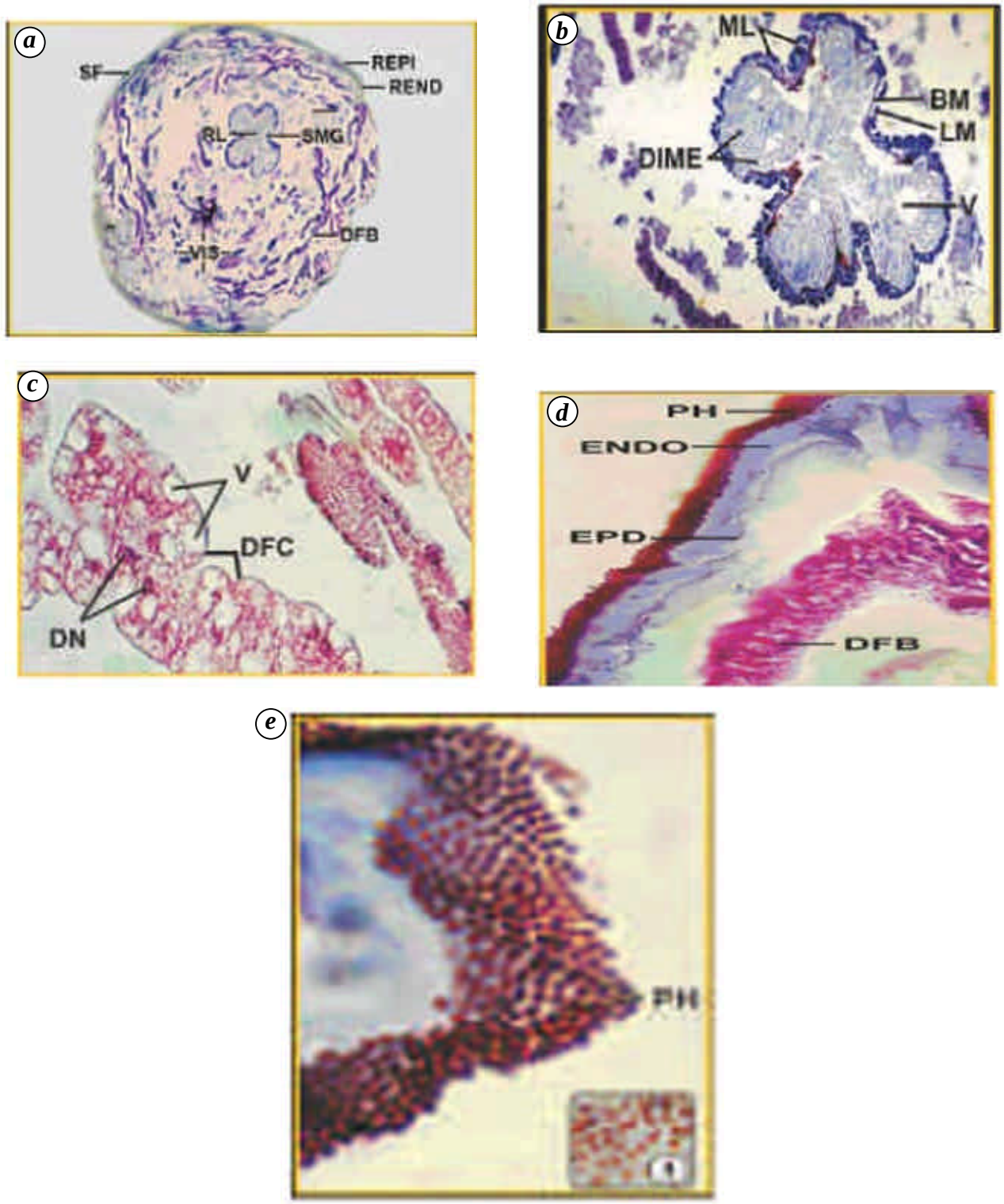

Figure 2. ***a***, Magnified view through body wall and midgut depicting thin and almost degenerated cuticle (DC), space formation (SF), infected connective tissues (ICT), degenerated midgut epithelium (DMGE), basement membrane (BM) and lumen (L); ×10. ***b***, Magnified view of midgut showing hypertrophied epithelial cells (HEC), longitudinal muscles (LM) circular muscles (CM) basement membrane (BM) with bacterial spore deposition (BS) in fat cells; ×40. ***c***, Highly magnified view of damaged fat body cells showing disintegrating nuclei (DN) with large vacuole formation (V) in the fat body cells (DFC) and complete loss of granular cytoplasm; ×40. ***d***, TS of body section showing damaged connective tissues (DCT) and fat cells (DFB). Midgut epithelial cells are completely degenerated (DMGE) forming lesions (L) along with degenerated longitudinal and circular muscular layer (LM and CM) and basement membrane (BM). Disintegrating trachea (DT) is also visible; ×100. ***e***, Magnified view of polyhedra (PH) deposited on the body wall. (Inset) Polyhedra; ×100.

soft and fragile, and liquefaction of body tissues inside the body cavity further adds to the tenderness of the infected insect. The chitinous cuticle of the insect which virtually covers all external surfaces, even extending through the foregut, hindgut and tracheal tubes constituting the first line of passive defence in insects[18].

The overall destruction of tissues led to liquefied contents inside the body cavity, giving the infected insect a turgid appearance. The infected larval body is laden with polyhedral occlusion bodies (POBs) which contain virions. Even a slight damage or disturbance of the integument released liquefied body fluid containing large number of POBs. This infected fluid further spread infection when healthy larvae came in contact with the fluid, causing autoinfection[15].

In the histomicrograph of infected insect, deposition of millions of Polyhedra was observed on the body wall, in the crypts of the body wall and inside the tissues (Figure 2 *c–e*). After being flooded with NPV, the protein content of the haemolymph is reduced and thus structural properties of the cuticle are affected leading to fragile skin and liquefied body fluid. The larvae become turgid and sluggish that they are unable to move.

To conclude, the histopathological studies have revealed that midgut epithelium is the principal target tissue for action of NPV. However, it was also observed in the histomicrographs that extensive tissue destruction occurred in the body and epithelium of the insect. These damages were from the centre towards the periphery, which was also evident by the development of various morphological and behavioural abnormalities developed after the insect was severely infected.

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Antiviral property of marine actinomycetes against White Spot Syndrome Virus in penaeid shrimps

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Aquaculture farms, particularly in Southeast Asia are facing severe crisis due to increasing incidences of White Spot Syndrome Virus (WSSV). Actinomycetes have provided many important bioactive compounds

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