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ORIGINAL ARTICLE

Anatomical and cytological studies on the cotton leaf worm *spodoptera littoralis* (Boisd.) larva infected with some bio-insecticides

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Abstract
This study was conducted to investigate the effect of recommended LC_{50} concentration of three commercial bio insecticides; (Protecto® (*Bacillus thuringiensis kurstaki*), Viruset® (*Spodoptera littoralis* NPV), Profect® (*Btk* & *Spli*NPV) on fat body and internal organs of the *spodoptera littoralis* larva treated with these bio insecticides. The treated larvae were dissected and examined under the converted microscope and transmission electron microscope. Results showed that the three compounds have significant effect on fat bodies and internal organs of larva. The greatest effect was observed by the profect compound followed by protecto and viruset. The combination of bacteria and virus increased the amount of fat body compared with suing virus and bacteria individually. Thus, our findings indicated that the combination of bacteria and virus increased the efficiency of each other.

Keywords Cotton leaf worm *spodoptera littoralis* · Microbial insecticides · Biological control · Fat body · Histology

1 Introduction

The cotton leaf worm, *Spodoptera littorals* (Bosid.) causes severe damage for cotton plants and subsequently leads to economic losses in cotton industry in Egypt. This pest develops resistance to the majority of chemical insecticides due to uncontrolled intensive use of these insecticides. Most chemical insecticides kill pest insects swiftly, but are also toxic to beneficial insects and other species in the agro ecosystem [1].To avoid unfavorable side effects of these insecticides on non-target organisms and environment, alternative safe and effective microbial insecticides have been initiated [1]. Insect viruses and entomopathogenic bacteria, fungi, and nematodes have been investigated as biological control agents of *S. littoralis* [2].Entomopathogenic bacteria, particularly Bacillus thuringiensis Berliner (*Bt*), is the most commonly used microbial insecticides. Cry toxins enjoy the advantages of highselectivity and the possibility of the application by sprays or transgenic plants [1]. According to Sanahuja et al. [3], *Bt*'s insecticidal activity is attributed to its proteinaceous crystals and vegetative insecticidal protein contents, which are produced during sporulation and vegetative growth phases, respectively Kumar et al. [4].

Baculovirus, the second most popular microbial insecticide, has been employed as a safe bio- insecticide because of its effectiveness and specificity as well as the presence of infectious protein crystals in it Zhang et al. [5]. The *Spodoptera littoralis* nucleopolyhedrovirus (SpliNPV) is a baculovirus that has been evaluated, registered, and applied for control of *S. littoralis*, as well as the fall armyworm, *Spodoptera frugiperda*, and the tobacco cutworm *Spodoptera. litura* in Africa, America and Japan Abdel-Khalik et al. [6], El-Sheikh; [7],Takatsuka et al. [8].

Similar to liver of vertebrates and hepatopancreas of crustaceans, the insect fat body (FB) has multiple metabolic functions and takes parts in the metabolisms of lipids, carbohydrates and proteins [9] and [10]. According to Wigglesworth [11] and Kritsky [12], it produces trehalose, desaminates and transamination of amino acids. Also, it removes calcium salts, urates, and other nitrogenated products from the hemolymph. The FB cells in Lepidoptera have the ability to phagocytose and take part in tissue remodelling during metamorphosis [11].

They have the ability to maintain a balance between the food resource and energy demands during the insect

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development. During the periods of insect actively feeding, FB accumulates molecules to be used in the periods of food leakage for developmental demands. Also, FB cells may change their activity in response to the nutritional and hormonal signals to supply the insect growth needs, metamorphosis and reproduction [13, 14]. However, these changes may also occur under various pathological and environmental conditions.

2 Materials and methods

2.1 Tested insects

The used insects are a laboratory strain of the cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) which had been reared in the laboratory without any exposure to chemicals and was obtained as egg masses from the Research Division of the cotton leaf worm, Plant Protection Research Institute, Dokki—Giza, Egypt.

2.2 Compounds

Three commercial bio insecticides were used in this investigation. All tested bio-agents were obtained as wettable powder produced by the Plant Protection Research Institute, Bio pesticide Production Unit, Dokki- Giza, Egypt. They were as follows:

2.2.1 a. Protecto® WP 9.4% (*Bacillus thuringiensis* var. *kurstaki*)

Bacillus thuringiensis (Kingdom: Eubacteria; Order: Bacillales; Family: Bacillaceae) is a gram-positive spore-forming bacterium that produces crystalline proteins called deltaendotoxins during its stationary phase of growth. This product is used at rate of 300 gm/feddan with LC_{50} value is 3.2×10^2 Iu/ml. Feddan is equal to 4200 square meter.

2.2.2 b. Viruset® WP 4% (*Spodoptera littoralis* ucleopolyhedrosis virus [*Spl*iNPV])

Nucleopolyhedrosis virus (NPV) is a double stranded DNA virus that belongs to baculoviruses subgroup (Family: Baculoviridae). The virus was isolated from diseased larvae and formulated to be used at rate of 300 gm/feddan and LC_{50} value of 1×10^2 PIBs/ml.

2.2.3 c. Profect® WP 5% + 2% (*Btk* + *Spl*iNPV)

This product is a mixture of 5% of *Bacillus thuringiensis* var. *kurstaki* and 2% of *Spl*iNPV. The product is used at rate of 400 gm/feddan and LC_{50} value is 5×10^3 Iu/ml and 1.6×10^2 PIBs/ml.

2.3 Preparing of insects for dissection

A newly ecdysed 2nd instar larva were fed on castor oil leaves treated with the determined LC_{50} of each compound for 24 h, then reared on untreated leaves until the symptoms of infection appeared. Infected larva were anesthetized and fixed in wax plate. Insects were dissected by cutting along their backs with an entomological scissors. The gut and other organ were examined then photographed under a stereo-microscope. Uninfected larva was used as control.

2.4 Specimen preparation for electron microscopy

After dissection of larva, mid gut was separated and preserved in a fixative solution. The resin embedded specimens were mounted in special holders which at the same time fit the microtome. Ultrathin sections were obtained using an ultra-cut E microtome equipped with a diamond knife (Diatome, Switzerland). Sections for TEM analysis were collected on carbon coated for more supports then stained with uranyl acetate and lead citrate [15]. After staining, sections were examined in a SEM electron microscope equipped with a ProScan slow scan CCD camera. Preparations and ultrathin sections were observed at 80 kV using a JEOL JEM -2100 at EM Unit, Mansoura University, Egypt.

3 Results and discussion

3.1 Morphological studies

3.1.1 Internal organs of untreated larvae

The Fat tissue (Fig. 1A–C) of *spopdoptera littoralis larva* was a whitish, spongy mass, suspended in the hemocoel and supported by the tracheal system and involved the organs of body cavity**.**

3.1.2 Internal organs of larvae treated with viruset compound

The treated larvae with viruset (Fig. 2A–C) appeared with reduced layer of fat tissue and tracheal system moderately attached to the gut.

3.1.3 Internal organs of larvae treated with Protecto compound

Larvae showed yellowish, reduced layer of fat tissue and less attached tracheal system to the gut (Fig. 3).

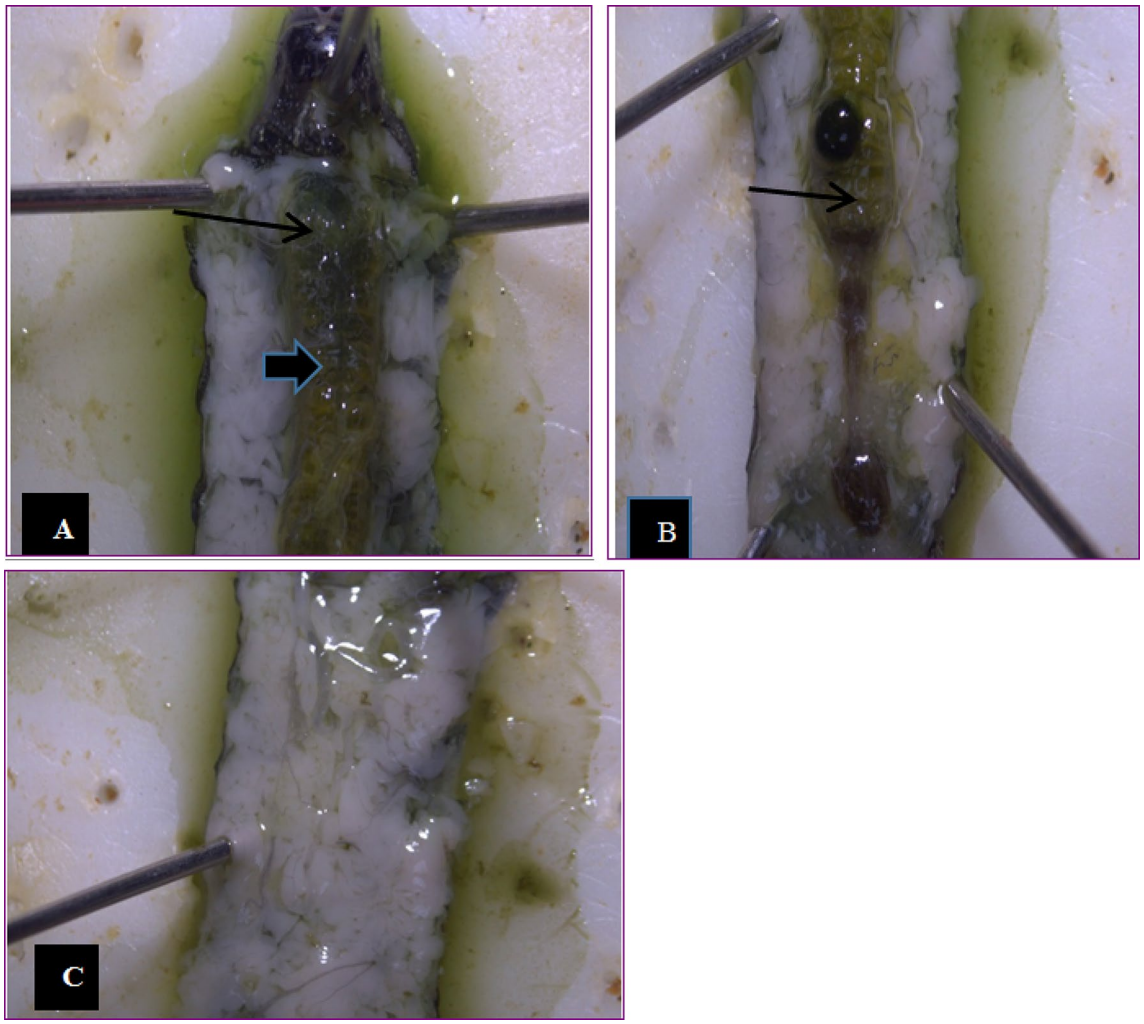

Fig. 1 Structural analysis of the internal tissues of *spodoptera littoralis* un treated larvae. **A** shows foregut (arrow) and midgut (arrow head). **B** shows hind gut (arrow). **C** shows fat tissue

3.1.4 Internal organs of larvae treated with profect compound.

The treated larvae showed a very thin layer of fat tissue and damaged tracheal system (Fig. 4).

3.2 Cytological studies

3.2.1 Ultra structure of mid gut of untreated larvae

See Fig. 5.

3.2.2 Ultra structure of mid gut of larvae treated with viruset compound:

See Fig. 6.

3.2.3 Ultra structure of mid gut of larvae treated with protecto compound

The cytoplasm of the epithelial cells become highly vacuolated as the vacuoles containing disintegrated glycogen granules (Fig. 7A–C). A part of epithelial cell in its way to be engulfed in the gut lumen (Fig. 7B). Bt invading microvilli of epithelial cell (Fig. 7B).

3.2.4 Ultra structure of mid gut of larvae treated with profect compound

See Fig. 8.

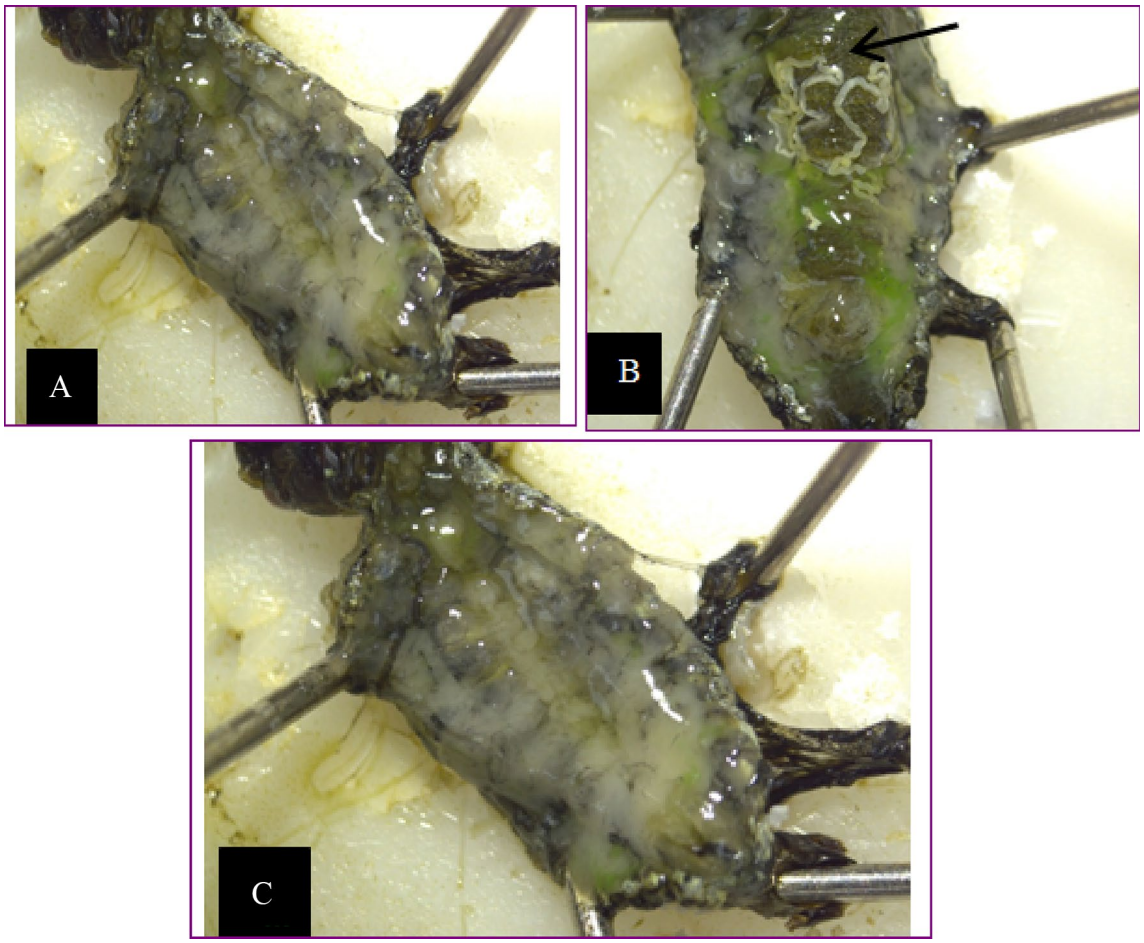

Fig. 2 Structural analysis of the internal tissues of *spodoptera littoralis* larvae infected with viroset compound. **A** shows foregu t(arrow) and hind gut (arrow head). **B** shows mid gut (arrow). **C** shows fat tissue

4 Discussion

Fat body, is a vital multi-functional tissue found in the visceral cavity of insect life stages [16, 17]. It carries out a variety of tasks, such as maintaining bacterial endosymbionts [18], storage of urate during development [19], synthesizing, releasing, and storing a variety of macromolecules [18]. It acts as a source of humoral factors, and role in immune functions [20].

The primary metabolites of insects include three major categories: lipids, proteins and carbohydrates. Our research showed that bacterial and viral infections may change these major categories in the host larvae. According to Arrese and Soulages [16], this effect may be associated with the insect fat body involved in synthesizing, storing and secreting lipids, proteins and carbohydrates. According to our study of the pathological morphology of fat-body tissues, viral and bacterial infection results in the destruction of the fat bodies, which may be one of the causes of the obvious changes in the host larva's major primary metabolite content. Schmid-Hempel [21], agreed with our conclusions and pointed out that virus infection results in the host's body physically losing nutrients, which are then given to the invading organisms to consume for their own growth and reproduction.

Our findings showed that larva treated with the profect compound, which is a mixture of bacteria and viruses, had a fatter body than larva treated with the bacteria and viruses separately. Dantzer, [22] agreed with us and provided an explanation for the observed rise in the lipid and protein contents of the infected larvae. They pointed out that the host's body causes stress and immunological responses to fight the invasive viruses. The host's metabolic rate rises as a result of these significant energy and resource demands.

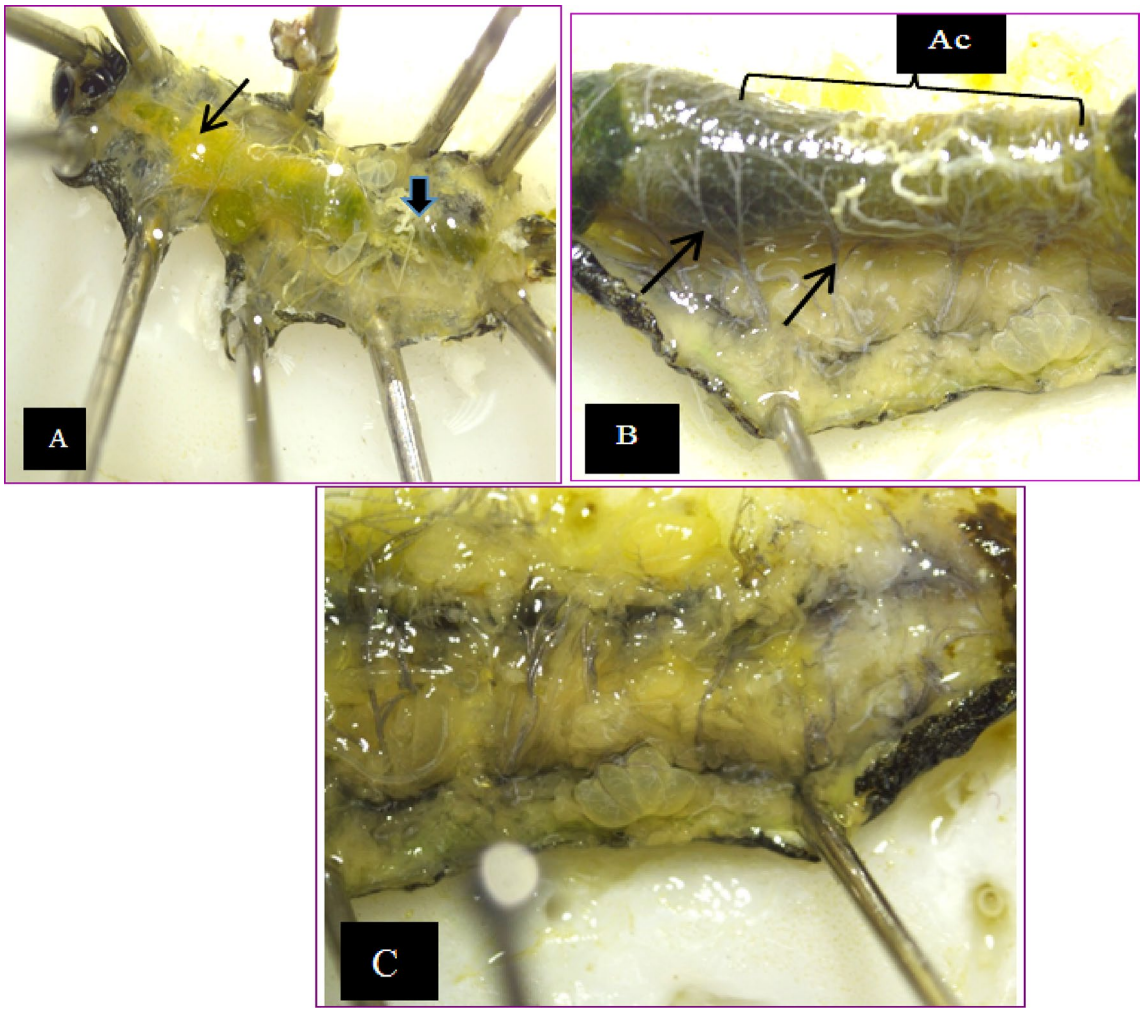

Fig. 3 Structural analysis of the internal tissues of *spodoptera littoralis* larvae infected with protecto compound. **A** shows foregu t(arrow) and mid gut (arrow head). **B** shows mid gut (arrow), Ac refers to alementary canal. **C** shows fat tissue of the insect

The midgut of the control larvae is composed of a single layer of columnar and goblet cells, as well as other epithelial cells, which are adhered to the muscular connective tissue by a basement membrane. Each epithelial cell has numerous microvilli. The cytoplasm contains a large number of organelles, such as mitochondria, lysosomes, free ribosomes, and rough endoplasmic reticulum. It has also, a spherical nucleus with a normal chromatin distribution and a typical nuclear envelope. A significant chamber known as the goblet cavity and an apical entry into the midgut lumen serve as the goblet cell's distinguishing features. Mitochondria is present in the numerous cytoplasmic extensions that line and expand into this cavity. The flattened nucleus of goblet cell is located in the basal region, just below the goblet cavity.

When the insect consumes a diet containing *Bt* formulation, the typical structure of the midgut of the larva changed. The *Bt* d-endotoxin crystals are dissolved in the midgut to produce one or more protoxin proteins. Specific receptors, which may be glycoproteins, are present in the brush border membrane of the midgut epithelium [23]. The Cry toxins may create pores that allow the passage of ions and small molecules like sucrose by inserting into the cell membrane. Osmotic inflow and cell lysis come from the disruption of the cell membrane's selective permeability. Thus the integrity of the epithelium is destroyed and the insect dies from starvation and/or septicemia [24].

In this study, the histopathological changes in the third instar *S. littoralis* larvae's midgut caused by *B. thuringensis* treatment were examined. The *Bt* action may be responsible for the histopathological alterations in the gut. These alterations include the loosening of the midgut columnar

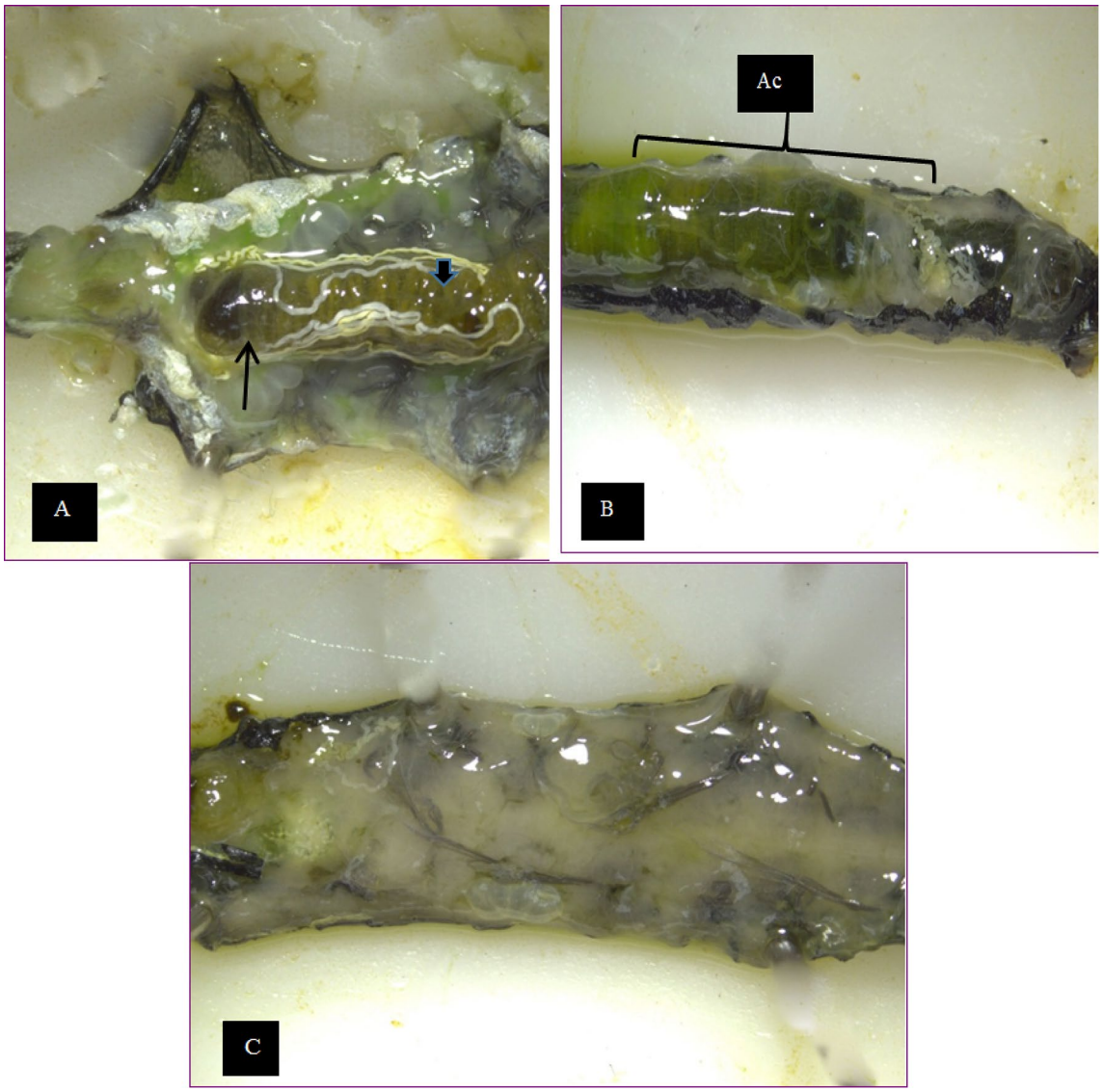

Fig. 4 Structural analysis of the internal tissues of *spodoptera littoralis* larvae infected with profect compound. **A** foregut (arrow) and mid gut (arrow head). **B** shows alementary canal (AC). **C** shows fat tissue of the insect

cells from the basement membrane and from one another. Cell vacuolization, microvilli destruction, and the passing of epithelial cell contents into the midgut lumen with nuclear migration to the apical membrane occur. Similar histopathological effects in midgut of *E. kuehniella* larvae treated with Cry toxins were found by [25].

In some cells, the microvilli have entirely separated from the apical surface. The contents of the epithelial cells are engulfed into the intestinal lumen once the microvilli detach. Dead cells detached from the epithelium have been seen in the mid gut lumen of numerous mosquitoes infected with several Plasmodium species, according to reports f Paskewitz et al. [26], Han et al. [27] Zieler and Dvorak [28]. They suggested that this might be a common response to various stressful situations.

Uncontrolled cell division, the development of rounded cells with little cytoplasm and a relatively big nucleus taking up the majority of the cell's surface area are one of the main effects of epithelial cells. Convoluted plasma membrane is noticed. The lysosomes separated from the cells leaving white vacuoles in its place. Nuclear sheath destruction, chromatin clumping and nuclear envelope shrinkage

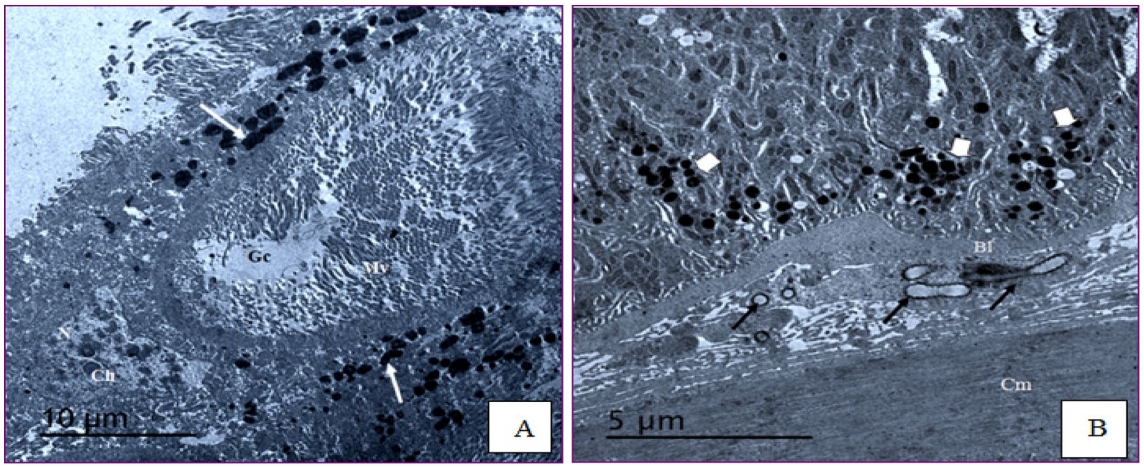

Fig. 5 Transmission electron micrograph of gut wall of *spodoptera littoralis* larvae. **A** Gc- goblet cell, Mv- microvilli, arrow-Glycogen granules, ch-chromatin, N-nucleus. The glycogen droplets increased in size and quantities, distributed all over the epithelial cells. Normal goblet cell appeared with normal microvilli. **B-**Arrow-trachea, Bl-basal lamina, Cm-circular muscle. The gut cell contained many glycogen granules. A thin basal lamina and a lot of tracheoles underneath the plasma membrane

were also noted. These conclusions are in agreement with those of Almeida et al. [29], Suryani et al. [30]. According to research by Barbeta et al. [31], epithelial cell displacement and hypertrophy from the basal lamina decrease the insect's ability to digest food. (Nasiruddin and Mordue [32] reported that, endoplasmic reticulum fragmentation, cytoplasm vacuolation, and microvilli disruption are the main reason of this hypertrophy.

Additionally, our findings demonstrated that columnar and goblet cells deteriorated and microvilli were damaged when the larvae consumed *Bt* endotoxin. Also, the goblet cells and epithelial cells dissolved, with its center engulfed into the gut lumen. The disruption of the midgut epithelial cell microvilli by *Bt* has been observed in other insect hosts by [33] and [34]. This result suggests that *Bt* toxins initially modify the microvillar membranes.

Similar reports of vacuolation of the *Bt*-affected midgut epithelial cells in lepidopterous hosts were made by Mathavan et al. [33] and Lane et al. [35]. They noted the extrusion of cytoplasmic globules from midgut cells that affected with *Bt*. According to Percy and Fast [36], Sutter and Raun [37], and Endo and Nishitsutsuji-Uwo [38], other lepidopteran species also exhibit similar extrusions. The nucleoplasm of the severely hypertrophied nucleus is scattered with folded and dispersed chromatin.

Some cells have microvilli that have entirely separated from the apical surface. The contents of the epithelial cells are absorbed into the gut lumen following the detachment of the microvilli. It is possible that dying epithelial cells shed into the lumen or phagocytosed by viable neighbour epithelial cells are part of a remodelling process of the midgut, as suggested for some mammalian organs [39]. However, dead cells detached from the epithelium have also been discovered in the midgut lumen of multiple mosquitoes carrying different Plasmodium species [26] and [28]. They suggested that this may be a typical reaction to various stress circumstances.

Baculoviruses (Bv) enter the haemocoel tissues through the basal lamina after occluded derived virions (ODVs) infect midgut cells. It's probable that the virus is telling columnar epithelial cells to release proteases at the basal lamina because the basal lamina is made up of protein. The midgut epithelium is not a primary site of viral replication for the majority of baculoviruses, but infected cells create the initial BV supply required to infect other organs in larvae. Infected midgut cells shed off as a virus defense mechanism, and new cells are produced to help in the recovery of gut. Allowing recovery of midgut epithelium enables the host to consume food, develop, and this allows the replication of virus.

According to Engelhard et al. [40], Kirkpatrick et al. [41], and Washburn et al. [42], the tracheal system is the main route by which viruses spread from the midgut. The tracheal system, or external insect respiratory system starts as apertures along sides of the insect called spiracles. Tracheal tubes extensively branch when they pass through spiracles and enter the haemocoel. These branches transition abruptly

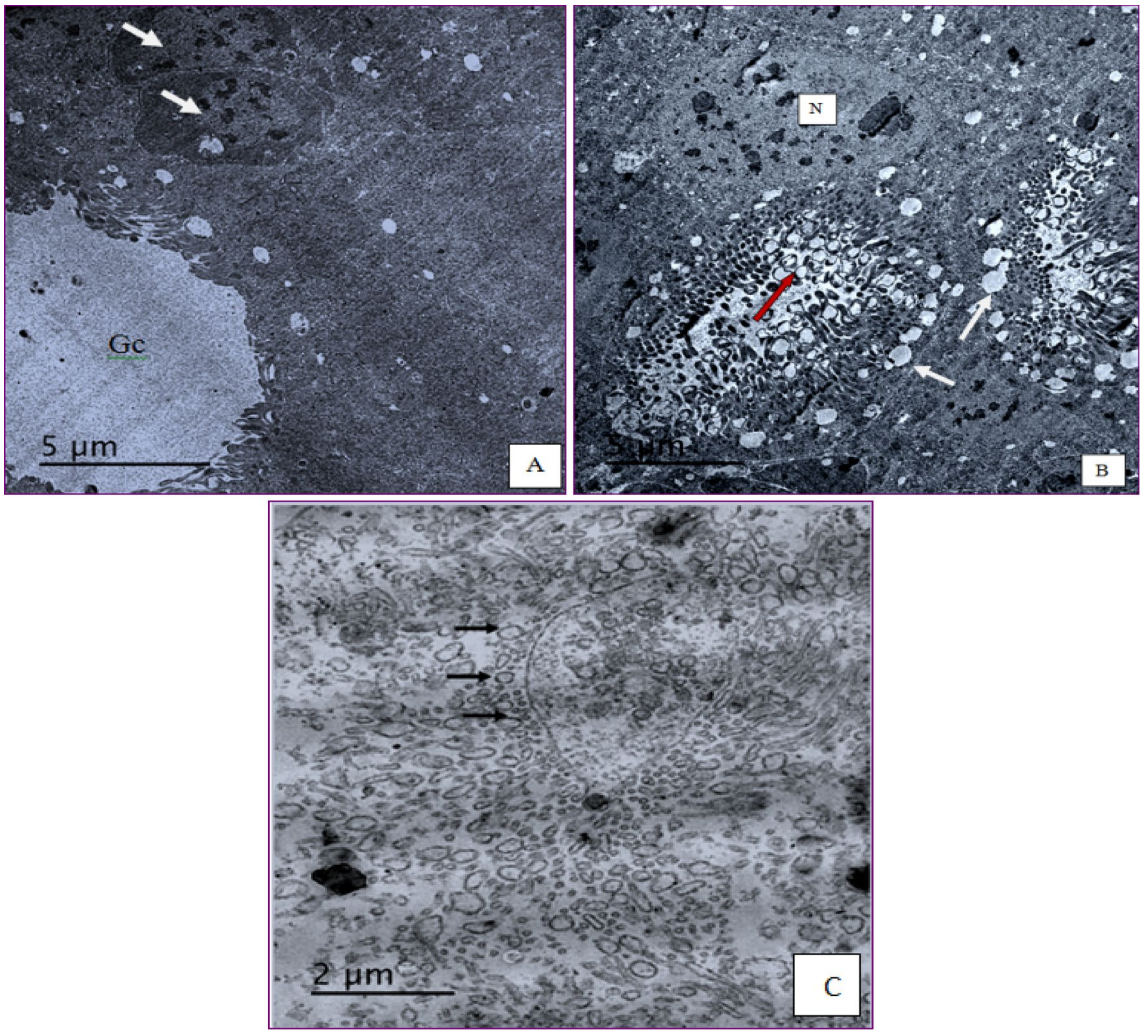

Fig. 6 Transmission electron micrograph of gut wall of *spodoptera littoralis* larvae treated with viruset compound. **A** Gc-highly infected goblet cell with fully degenerated microvilli, arrow- degenerated nucleus. **B** Red arrow—fully formed polyhedral of the virus, white arrow- processed polyhedral, N—nucleus of epithelial cell. **C** Arrow—polyhedra stuff the gut wall of epithelial cell. Late stage of virus infection resulting in complete hydrolysis of the epithelial gut wall and formation of liquefied gut content

into a network of very small tubes called tracheoles. Tracheoles impregnate nearly every tissue including the midgut epithelium. When a tracheolar cell infected by a midgut cell-derived BV, the BV progeny will gain access to the hemocoel's deep tissues.

The fat body which serves as the liver of the insect is an important component of baculovirus replication [43]. I is responsible for sugar storage and lipid metabolism in insect. In the lepidopteran larva, the fat body is an amorphous and protuberant organ running throughout the insect. The tissue is highly accessible to BV and the energy-rich cells of the fat body are ideal for producing abundant virus progeny. The fat body becomes full up with OBs, giving an opalescent, puffy appearance to the larvae just before it dies. Exploiting the inner tissues of the insect host gives the virus the advantage of being able to produce enormous numbers of progeny. Most baculoviruses release OBs from the host by tissue liquefaction and the rupture of cuticle after death. As the liquefied remains ooze out from the dead host, OBs are broadly dispersed along food surfaces that are eaten by a new host.

Within 30 min of infection, viral early genes are expressed, and its protein products, together with virion protein and begin to enable DNA replication [44].The structural

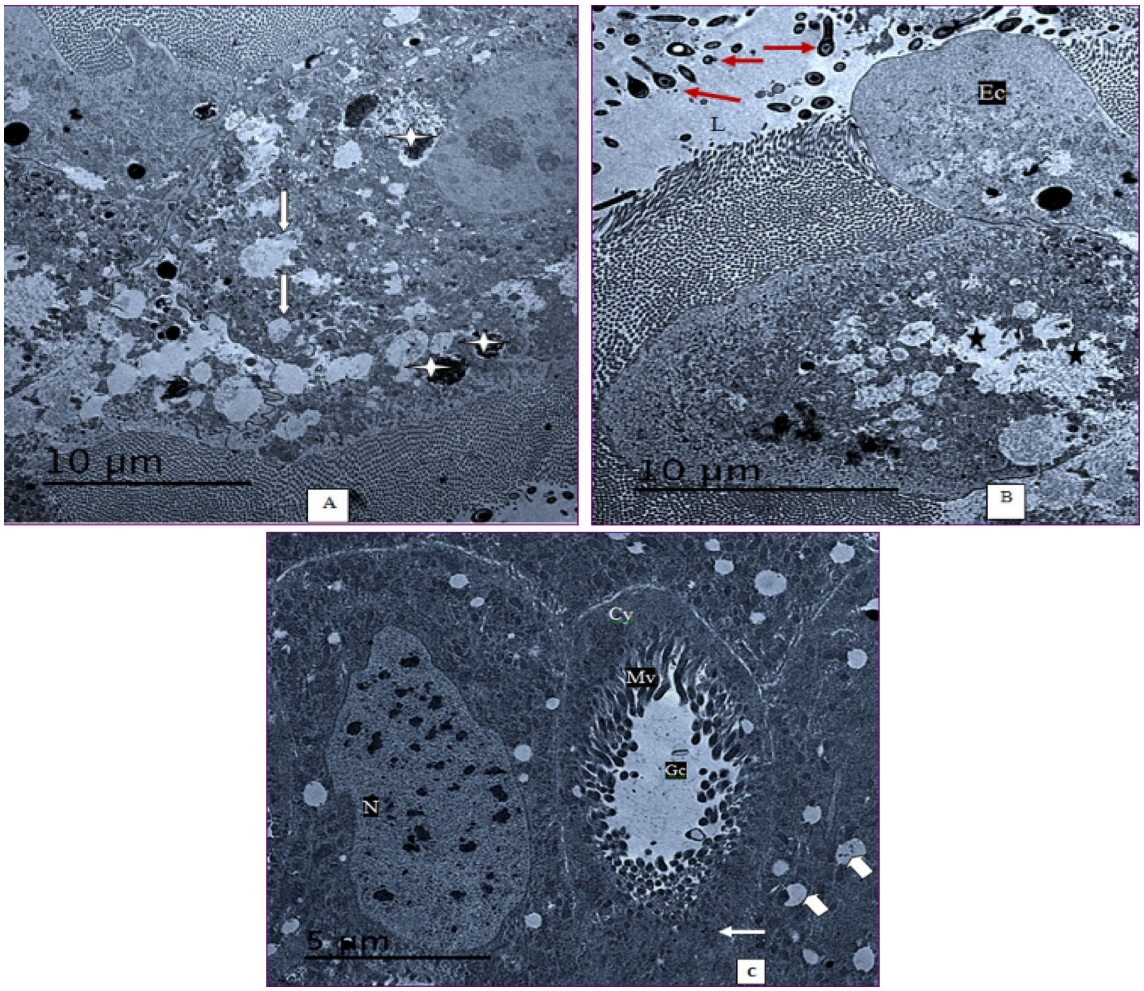

Fig. 7 Transmission electron micrograph of sector of gut wall of *spodoptera littoralis* larvae treated with protecto compound. **A** Arrows-Glycogen (lipid) vacuoles. Stars—glycogen vacuole in different stages of degeneration. **B** Red arrows-sections of bacterial cells. Ec- part of epithelial cell in its way to be engulfed in the gut lumen. Star- disintegrated glycogen vacuole. **C** Gc-goblet cell. Arrow- sections of Bt accumulated in the gut lumen. Arrow head- disintegrated glycogen vacuole.Mv- microvilli of epithelial cells invaded with Bt. N- hypertrophied nucleus of epithelial cell having dispersal chromatin granules all over the nucleoplasm

alteration of the nucleus, known as nuclear hypertrophy, results in an enlarged nucleus. This is in line with our observations, which demonstrated that the nucleus hypertrophy and chromatin was dispersed throughout the nucleoplasm.

When *Anticarsia gemmatalis* is exposed to squamocin, midgut cells undergo autophagy, which results in damage to microvilli and severe cytoplasmic vacuolization. Our findings matched what Costa et al. [45] discovered in the midgut digestive cells of *A. aegypti* larvae exposed to squamocin. According to Gaban et al. [46], the mid gut is severely affected when *A. aegypti* larvae treated with novel chemical insecticide demonstrate high secretory activity and droplets discharged to the gut lumen from the apical region of the cell.

When combining the virus and bacteria together a significant effect on the mid gut epithelium and visceral muscle occur. Columnar and goblet cells are totally broken down. After the microvilli have been completely separated from the cell, lysis takes place. The same results observed by Knaak and Fiuza [47], who noted the destruction of microvilli and lyses of *Anticarsia gemmatalis* cells treated with *Bacillus thuringensis* and nuclear polyhedrosis virus. They demonstrated that there were no alterations in the intestinal cells of *A. gemmatalis* larvae in the individual treatments with *Bt.k.* and *Ag*NPV up to 6 h after application. After 6 h, *Btk* and *AgNPV* together already showed significant cytoplasmic vacuolization, leading to cellular disorganization. Salama et al. [48] and Mahmoud et al. [49] supported our findings. They clarified that following *B.t* and NPV treatment, vegetative cells and spores of *B.t* penetrated the midgut epithelial cells, followed by penetration of the virus. The potential entry of *B. t* and the

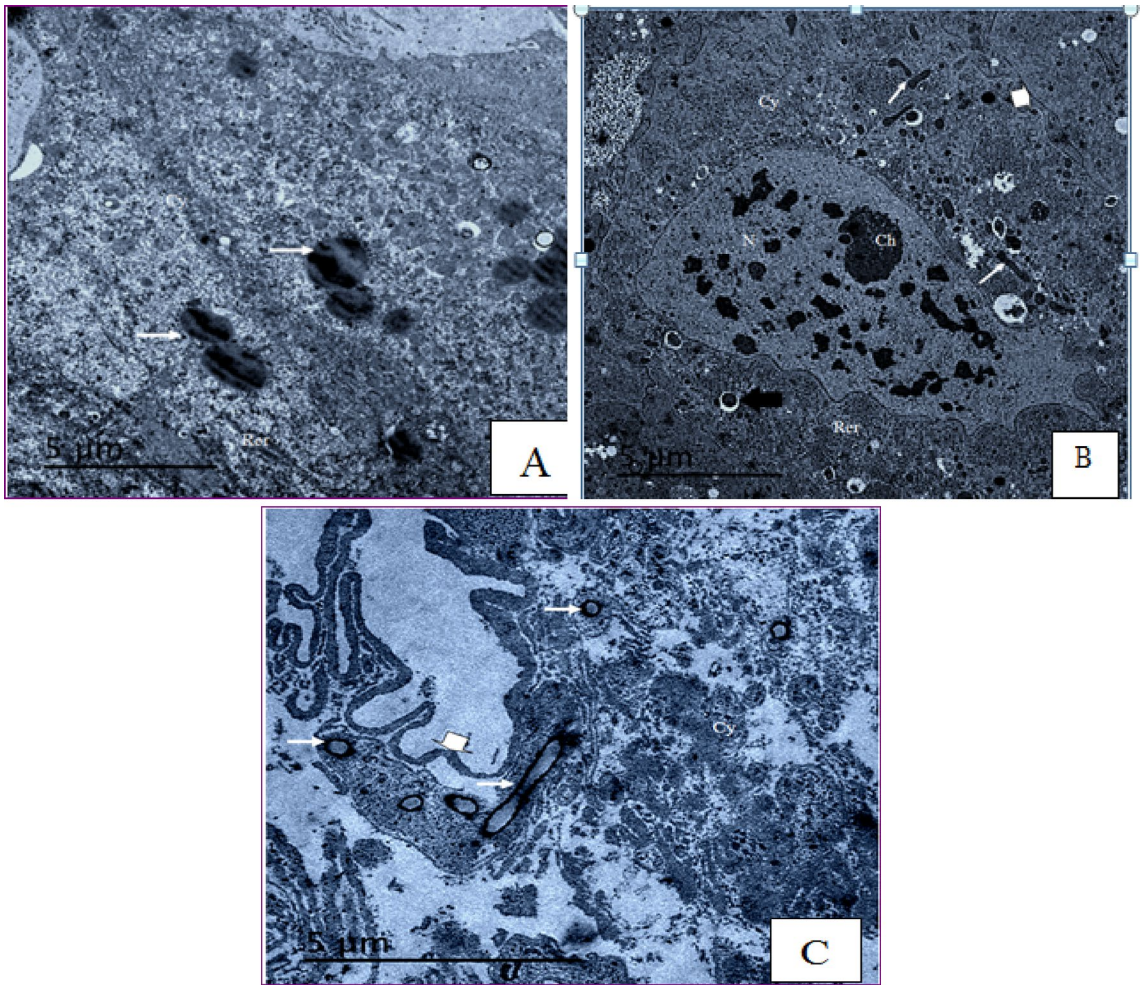

Fig. 8 Transmission electron micrograph of sector of gut wall of *spodoptera littoralis* larvae treated with profect compound. **A** Arrow-glycogen granules. **B** arrow-BT in the cytoplasm, thick arrow- glycogen granules. N-hypertrophied nucleus with chromatin granules scattered all over the nucleoplasm**. C**-arrows-trachea, cy-cytoplasm, thick arrow-basal lamina

ensuring release of toxin led to a partial breakdown of the cell organelles, which made it easier for the virus to enter from nearby fat tissues**.** Magholli et al. [50] explained that the synergistic effects of the two pathogens, *B.t* and NPV, on *Heliothis armigera* may be due to the delay in pre-pupal stage.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by the authors. The first draft of the manuscript was written by [SMSB] and all authors commented on previous versions of the manuscript. The authors read and approved the final manuscript.

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Data availability The datasets collected and/or analyzed during the current study are available from the corresponding author on request.

The corresponding author had full access to all the data in the study and takes responsibility for the integrity of the data.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

1. Heckel DG (2020) How do toxins from Bacillus thuringiensis kill insects? An evolutionary perspective. Arch Insect Biochem Physiol 104:1–12
2. Hajek AE, Shapiro-Ilan DI (2018) Ecology of invertebrate diseases. Wiley, Hoboken
3. Sanahuja G et al (2011) Bacillus thuringiensis: a century of research, development and commercial applications. Plant Biotechnol J 9(3):283–300
4. Kumar P, Kamle M, Borah MDK, Sharma B (2021) *Bacillus thuringiensis* as microbial biopesticide: uses and application for sustainable agriculture. Egypt J Biol Pest Control 31:95. https://doi.org/10.1186/s41938-021-00440-3
5. Zhang S, Wu F, Li Z, Lu Z, Zhang X, Zhang Q, Liu X (2015) Effects of nucleopolyhedrovirus infection on the development of *Helicoverpa armigera* (Lepidoptera:Noctuidae) and expression of its 20-hydroxyecdysone- and juvenile hormone-related genes. Florida Entomologist 98:682–689
6. Abdel-Khalik LE, El-Sheikh E, Ragheb D, Ashour M (2017) Efficacy and virulence of *Spodoptera littoralis* nucleopolyhedrovirus on *S littoralis* larval feeding and susceptibility. Zagazig J Agricult Res 44:261–271
7. El-Sheikh E (2015) Efficacy of Spodoptera littoralis nucleopolyhedrovirus on Spodoptera frugiperda (J.E. Smith) and Spodoptera exigua (Hübner): virulence biological effects and inhibition of juvenile hormone esterase. Egypt J Biol Pest Control 25(3):587–595
8. Takatsuka J, Okuno S, Nakai M, Kunimi Y (2016) Genetic and phenotypic comparisons of viral genotypes from two nucleopolyhedroviruses interacting with a common host species *Spodoptera litura* (Lepidoptera: Noctuidae). J Invertebr Pathol 139:42–49. https://doi.org/10.1016/j.jip.2016.07.009
9. Chapman RF (1998) The insect: structure and function. University Press, Cambridge
10. Eldridge FB, Edman DJ (2004) Medical Entomology: a text book on public health and veterinary problems caused by arthropods. Springer, London
11. Wigglesworth VB (1984) The principles of insect physiology, 8th edn. Chapman and Hall, London
12. Kritsky G (2002) A survey of entomology. Universe, New York
13. Cunha MAS, Cruz-Landim C (1983) Modificações Histológicas e Histoquímicas do Corpo Gorduroso de Rainhas de Atta sexdens rubropilosa Forel (Hymenoptera: Formicidae) durante o Primeiro Ciclo Reprodutivo. Act Biol. 12(1,2,3,4):11–12
14. Gullan PJ, Cranston PS (2005) The insects on outline of entomology, 3rd edn. Blackweel Publishing, London
15. Reynolds ES (1963) The use of lead citrate at a high pH as an electron opaque stain in electron microscopy. J Cell Biol 17:208–212
16. Arrese EL, Soulages JL (2010) Insect fat body: energy, metabolism, and regulation. Annu Rev Entomol 55:207–225
17. Hoshizaki DK, Gibbs AG, Bond ND (2012) Fat body, R.F. Chapman's the insects: structure and function, 5th edn. Cambridge University Press, New York, pp 132–145
18. Costa-Leonardo AM, Laranjo LT, Janei V, Haifig I (2013) The fat body of termites: functions and stored materials. J Insect Physiol 59:577–587
19. Park MS, Park P, Takeda M (2013) Roles of fat body trophocytes, myetocytes and urocytes in the American cockroach, Periplaneta americana under starvation conditions: an ultrastructural study. Arthropod Struct Dev 42:287–295
20. Gillespie JP, Kanost MR, Trenczek T (1997) Biological mediators of insect immunity. Annu Rev Entomol 42(1):611–643
21. Schmid-Hempel P (2011) Evolutionary parasitology:the integrated study of infections, immunology, ecology, and genetics. Oxford University Press
22. Dantzer R (2004) Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. Eur J Pharmacol 500(1–3):399–411
23. Knowles BH, Dow JAT (1993) The crystal δ-endotoxins of Bacillus thuringiensis: models for their mechanism of action in the insect gut. BioEssays 15:469–476
24. Boukedi H, Khedher SB, Abdelkefi-Mesrati L, Van Rie J, Tounsi S (2018) Comparative analysis of the susceptibility/tolerance of Spodoptera littoralis to Vip3Aa, Vip3Ae, Vip3Ad and Vip3Af toxins of Bacillus thuringiensis. J Invertebr Pathol 152:30–34. https://doi.org/10.1016/j.jip.2018.01.006
25. Liu L, Chen Z, Yang Y, Xiao Y, Liu C, Ma Y, Soberón M, Bravo A, Yang Y, Liu K (2018) A single amino acid polymorphism in ABCC2 loop 1 is responsible for differential toxicity of Bacillus thuringiensis Cry1Ac toxin in different Spodoptera (Noctuidae) species. Insect Biochem Mol Biol 100:59–65. https://doi.org/10.1016/j.ibmb.2018.06.004
26. Paskewitz SM, Brown MR, Collins FH, Lea AO (1989) Ultrastructural localization of phenoloxidase in the Midgut of refractory anopheles gambiae and association of the enzyme with encapsulated plasmodium cynomolgi. J Parasitol 75(4):594–600
27. Han YW, Shi W, Huang GT, Kinder Haake S, Park NH, Kuramitsu H, Genco RJ (2000) Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells. Infect Immun 68(6):3140–3146
28. Zieler H, Dvorak JA (2000) Invasion in vitro of mosquito midgut cells by the malaria parasite proceeds by a conserved mechanism and results in death of the invaded midgut cells. Proc Natl Acad Sci USA 97(21):11516–11521
29. Almeida GE, Zanuncio JC, Pratissoli D, Polanczyk RA (2014) Cytotoxicity in the midgut and fat body of Anticarsia gemmatalis (Lepidoptera: Geometridae) larvae exerted by neem seeds extract. Invertebr Surviv J 11:79–86
30. Suryani AI, Hariani N, Majid AF, Amalia D (2020) Histological changes in the midgut of Spodoptera litura larvae exposured by the extract of Mirabilis jalapa leaves. IOP Conf Ser Earth Environ Sci 484:012107
31. Barbeta BL, Marshall AT, Gillon AD, Craik DJ, Anderson MA (2008) Plant cyclotides disrupt epithelial cells in the midgut of lepidopteran larvae. Proc Natl Acad Sci U S A 105(4):1221–1225. https://doi.org/10.1073/pnas.0710338104. (**Epub 2008 Jan 17. PMID: 18202177; PMCID: PMC2234119**)
32. Nasiruddin M, Mordue (luntz) AJ (1993) The effect of azadirachtin on the midgut histology of the locusts, Schistocerca gregaria and locusta migratoria. Tissue Cell 25(6):875–884
33. Mathavan S, Sudha PM, Pechimuthu SM (1989) Effect of Bacillus thuringiensis israelensis on the midgut cells of Bombyx mori larvae: A histopathological and histochemical study. J Inver tebr Pathol 53:217–227
34. Iman I (2018) Histological Effect of Bacillus thuringiensis Isolate against Pink Bollworm Larval Midgut, Pectinophora gossypiella (Saund). J. Plant Prot Pathol Mansoura Univ. 9(12):803–806
35. Lane NJ, Harrison JB, Lee WM (1989) Changes in microvilli and golgi-associated membranes of lepidopteran cells induced by an insecticidally active bacterial-endotoxin. J Cell Sci 93:337–347
36. Percy J, Fast PG (1983) Bacillus thuringiensis crystal toxin: Ultrastructural studies of its effect on silkworm midgut cells. J Invertebr Pathol 41:86–98
37. Sutter GR, Raun ES (1967) Histopathology of European-cornborer larvae treated with Bacillus thuringiensis. J Invertebr Pathol 9(1):90–103

38. Endo Y, Nishiitsutsuji-Uwo J (1980) Mode of action of Bacillus thuringiensis δ-endotoxin: Histopathological changes in the silk worm midgut. J Invertebr Pathol 36:90–103
39. Fadok VA (1999) Clearance: the last and often forgotten stage of apoptosis. J Mammary Gland Biol Neoplasia 4(2):203–211. https://doi.org/10.1023/a:1011384009787. (**PMID: 10426399**)
40. Engelhard EK, Kam-Morgan LNW, Washburn JO, Volkman LE (1994) The insect tracheal system: a conduit for the systemic spread of Autographa californica M nuclear polyhedrosis virus. Proc Natl Acad Sci USA 91(8):3224–3227
41. Kirkpatrick BA, Washburn JO, Engelhard EK, Volkman LE (1994) Primary infection of insect tracheae by Autographa californica M nuclear polyhedrosis virus. Virology 203(1):184–186
42. Washburn JO, Kirkpatrick BA, Volkman LE (1995) Comparative pathogenesis of Autographa californica M nuclear polyhedrosis virus in larvae of Trichoplusia ni and Heliothis virescens. Virology 209:561–568
43. Dean RL, Locke M, Collins JV (1985) Structure of the fat body. In: Kerkut GA, Gilbert LI (eds) Comprehensa insect physiology, biochemistry and pharmacology, vol 9. Pergamont Press, London, pp 155–210
44. Chisholm GE, Henner DJ (1988) Multiple early transcripts and splicing of the Autographa californica nuclear polyhedrosis virus IE-1 gene. J Virol 62(9):3193–3200
45. Qi Y, Wang S-S, Li L-L (2023) IE1 of autographa californica multiple nucleopolyhedrovirus activates low levels of late gene expression in the absence of virus RNA polymerase. Microbiol Spectr 11(1):e03432-e3522. https://doi.org/10.1128/spectrum.03432-22
46. Costa M, De Paula S, Martins G, Zanuncio J, Sant'Ana A, Serrão J (2016) Multiple modes of action of the squamocin in the midgut cells of *Aedes aegypti* larvae. PLoS One 11:e0160928. https://doi.org/10.1371/journal.pone.0160928
47. Gaban C, Arruda E, Dourado D, Silva L, Paulo N, Cabrini I (2015) Morphological changes in the digestive system of *Aedes aegypti* L. Induced by [Cu(EDTA)]2- complex ions. J Mosq Res. https://doi.org/10.5376/jmr.2015.05.0021
48. Knaak N, Fiuza LM (2005) Histopathology of Anticarsia gemmatalis Hübner (Lepidoptera; Noctuidae) treated with Nucleopolyhedrovirus and Bacillus thuringiensis serovar kurstaki. Braz J Microbiol 36:196–200
49. Salama H, Sharaby A, Magd El-Din M (1993) Mode of action of Bacillus thuringiensis and nuclear polyhedrosis virus in the larvae of *Spodoptera littoralis* (Boisd.). Int J Trop Insect Sci 14(4):537–543. https://doi.org/10.1017/S1742758400014235
50. Mahmoud DM, Abd El-Bar MM, Abdul Aziz Radi MH (2012) Combined effect of local isolate *Spodoptera littoralis* nucleopolyhedrosis virus and *Bacillus thuringiensis* on *Culex pipiens* L. larvae (Culicidae: Diptera). J Basic Appl Zool 65(1):74–78. https://doi.org/10.1016/j.jobaz.2012.10.007
51. Magholli Z, Marzban R, Abbasipour H et al (2013) Interaction effects of *Bacillus thuringiensis* subsp. *Kurstaki* and single nuclear polyhedrosis virus on *Plutella xylostella*. J Plant Dis Prot 120:173–178. https://doi.org/10.1007/BF03356471