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Assessment of Mode of Action and Histopathological Changes Induces by *Bacillus thurengiensis*. in Various Tissues and Organs of *Spodoptera littoralis* Larvae

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***Abstract*— The present study was devoted to elucidate the mode of action and histopathological effects of the spore δ-endotoxin complex of *B.t.* var aizawai on the larvae of the cotton leaf worm *Spodoptera littoralis*.The results obtained has clearly shown that the consequence of symptoms of infection could be divided in morphologically distinct four stages. Furthermore, the results of this investigation have indicated that the sequence of symptoms starts with cessation of feeding followed by movement sluggishness, vomiting and diarrheae, excessive sluggishness, complete paralysis and finally death of the insect. On the other hand, the histopathological effects of the endotoxin observed in the dissected insect fed on diet containing the toxin were followed periodically. The results have clearly demonstrated marked histopathological alterations in the midgut epithelium, layers and clumping of both exo-and endocuticle of the integument. Furthermore, the uptake of bacterial δendotoxin has caused a marked degeneration of the nerve cells of the fourth abdominal nerve ganglion. The nerve cells and fibers were partially destroyed. In addition a notable destruction and vacuolation of the fat body cells became evident and the fat tissues became soft and easily crushed as compared to those of the healthy insect.**

***Index Terms*— *Spodoptera littoralis*, *Bacillus thurengiensis*, Larval tissues, Histological changes.**

I. Introduction

Introduction. It is assumed that the susceptible host to as given type of δ-endotoxin has in the gut some protolytic enzymes capable of breaking down the crystal into its subunits that some of which are toxic to that insect species. when the susceptible insect eats δ-endotoxin, certain hestological changes take place. First, the gut becomes paralyzed accompanied with significant damage to the intestinal wall. These changes are followed by a toxemia and/or septicemia taking place sometimes after the gut paralysis. The earliest report on the mode of action of this toxin is that of Heimple and Angus 1959 Who noted that the intoxication of the susceptible insect follows within minutes after crystals ingestion. The increase in the bacterial concentration showed prolongation in the larval period and a decrease in the pupal weight (Sareen *et al*. 1983), they reported that larvae as *S.littoralis* consumed less amount of green foliage treated with the organism as compared to control. The effect of δ-endotoxin of *B.t* on the gut movement of the silkworm, *Bombyx mori* was studied by Hukhara *et al*. (1984), they reported that pre oral administration of toxic crystals of *Bt* to larvae if the silkworm resulted in the inhibition of rhythmic contractile movements of the gut. They found that the paralysis began in the second-fourth of the midgut and the affected region was extended to the anterior midgut except for the end. They reported also that the administration of activated toxin resulted in an earlier manifestation of paralysis in a wider portion of the midgut. Luethy and Studer (1986) found that the breakdown of the gut epithelium is the primary cause for the lethal action of the δ-endotoxin of *Bt* var. israelensis. It appears that the toxin was cytolytic and acted by disruption of membrane permeability system. Singh *et al*. (1986) studied the toxic action of *Bt* var. israelensis an *Aedes aegypti* in vivo. They found that the skeletal muscles swell, the plasma membrane separates from underlying myofibrils, and mitochondria lose their structural integrity. They suggested that necrosis of skeletal muscles is the principal cause of paralysis of *Bt* treated insects. Mohsen *et al.* (1987) studied the histological changes in the midgut of 4 th instar larvae of *Culex quinquefasciatus* 24 hrs after exposure to spores of *Bt* H-14 and *B. sphaericus* 2362 as ingestion by larvae. They reported notable hypertrophy, hyperplasia and multilayered epithelial cells of the midgut. They found that the ingestion of *B.sphaericus* 2362 resulted in separation epithelial cells but both bacteria resulted in rupture, lyric vacuoles and varying degree of sloughing. The mechanism of action and receptor binding of a dual specificity *Bt*. var Aizawai ICI-δ-endotoxin using insect cell culture. They proposed that the initial interaction of toxin with a unique receptor determines the specificity of the toxin, following which cell death occurs by a mechanism of colloid osmotic lyses. The aim of this investigation is to demonstrate the response of different types of tissues in *S. littoralis* to the pathological action of the entomopathogenic bacterium *Bt* Aizawai HD-282. All changes that observed was recorded by the light microscope.

II. Materials & Methods

1- Organisms used . The *B.t* var.Aizawai culture number HD-282 serotypeH7 Biotype VII . These culture were originally obtained from cotton insect research institute , USA Brownsville Texas, USA and agriculture Canada research station, Manitoba, Canada.

2- Insect culture breeding . Astandard laboratory culture of the cotton leaf worm *S. littoralis* was maintained in the laboratory on leaves of castor oil . For this purpose, egg-masses were dipped in 1% formaldehyde for 2 minutes and then left to dry. Every egg-mass was kept in a clean glass

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jar (250ml.) containing fresh castor oil leaves after being washed with water and dried. The hatched larvae usually oriented themselves towards the leaves where they fed; fresh leaves were supplied daily. Third instar larvae were transferred in groups of ten to bigger clean jars (1 Liter) containing a layer of saw dust at the bottom and provided with fresh leaves to avoid overcrowding. The jars used in rearing were always washed with soap and 5% formaldehyde and dried in order to prevent any possible contamination with virus infection. The pupae were collected daily and placed in wooden cage (70x90x50cm.) with wire gauze sides (2mm mesh) and a layer of saw dust at the bottom. After emergence the adults were fed on 10% honey solution. Leaves of *Nerium oleander* were provided as oviposition sits. Egg-masses were transferred to glass jars where they were left to hatch. Appropriate feeding was then provided until the newly hatched larvae reached the second instar of age. Such larvae of this stage were used in the bioassay of the *Bt* var aizawai HD282.

3- Bioassay of *B.t* aizawai HD282. The bioassay procedure described by Dulmage *et al*. (1971) was applied but the castor oil leaves was used instead of the artificial diet (Morris, 1991). The leaves were cut into small discs(2cm diam.), then dipped into the tested *Bt* solution (5000 µg Bt aizawai HD 282/ ml distilled water + two drops of Tweem 80), then the discs were dispensed in small plastic cups. The bioassay was carried out using 10 newly second instar larvae of *S.littoralis* in each replicate The cubs infested with the larvae were incubated at 26± 2ºc for seven days . The still alive larvae were taken for histological studies. For the control the hatched larvae were fed on castor oil discs dipped into distilled water with two drops of Tween 80 only without *Bt.*

4- Histological studies. The treated larvae of *S. littoralis* were isolated daily from the laboratory colony fed on castor oil plant leaves treated with *Bt* . The obtained treated larvae were fixed in alcoholic Boun' s fluid and this continued till the seven day of treatment. Fixation was made for 48-72 hours. The fixative was washed out by passing the material in 70% ethanol for several times. The material was then dehydrated by passing in ascending series of ethanol followed by xylol and then embedded in paraffin wax and prepared for sectioning. Transverse sections were cut at thickness of 6 microns and stained with differential double stain Ehrilich' s haematoxylin and eosin section of untreated larvae of the same age were also prepared in the same manner for comparing with the treated ones. The aim of this investigation to demonstrate the response of the different types of tissues in *S. littoralis* larvae to the pathological action of entomopathogenic bacterium *Bt* var. aizawai HD-282.

III. Results & Discussion

A. *Symptoms of B.t infection.*

The consequence of symptoms of infection by *Bt* var. aizawai HD-282 were recorded in the larvae of cotton leafworm *S.littoralis* from the time of initial administration to larval death. The succession of symptoms can be divided into the following stages.

1-The larvae appear and act normally in all aspects. They look active and feed normally, but on the second day after feeding, the larvae diminish feeding. 2- In the second stage , the locomotion of the larvae slows down. The midgut of the live insect dissected in physiological saline solution shows normal peristalsis. 3- In the third stage, the larvae become so sluggish, turn black in color with signs of vomiting and diarrhea. The larvae cannot return normal if they turn upside down. All appendages (mouth parts and legs) show a reaction when stimulated with a needle, but there is no spontaneous movement. 4- In the fourth stage, the reflex movement disappear, with complete paralysis of the larvae. The fore and hind gut show contraction when the larvae are dissected in saline solution. These finding are in general agreement with those reported in *Bombyx mori* (Nishisutsuji-Uwa and Endo, 1980) and in *S.littoralis* by (Salama *et. al* 1984&1991). This indicate that the larvae of *S.littoralis* die as a result of treatment with *Bt.* with a sequence of the following symptoms: Cessation of feeding, sluggishness, vomiting and diarrhea, excessive sluggishness, complete paralysis and loss of reflex movement , then finally death.

B. *Histopathlogical effects of Bt var. aizawai HD-282 in S.littoralis.*

The investigations demonstrated the response of the different types of tissues in S. littoralis to the pathological action of the entomopathogenic bacterium *Bt* var.aizawai HD-282. The changes that occur can be shown as follows:

C. *1- Effect on the midgut.*

The midgut is a three-layer portion of the entire gut. It is cylindrical, repeatedly constricted and is comprised of an outer muscular layer (Masculosa), a basement lamina and monolayer of three kind of epithelial cells. These are the columnar cells, goblet cells and a number of small basal regenerative cells near the base of the other kind of cells (Fig.1). The columnar cells bear numerous microvilli at their apices forming a striated border in the periphery of the gut lumen. This border is probably responsible for digestion, absorption and secretion. The goblet cells are calyx shaped and occur between the columnar cells. Each cell contains a large goblet cavity in its central part. Numerous microvilli extend into the goblet cavity, these are apparently responsible for the transport of potassium ions into the intestine (Griego *et al*.,1979). Regenerative cells are small cells oval or circular found singly with prominent nuclei.

D. *Midgut- epithelium of infected larvae.*

The light microscope examination shows the rapid and vigorous destruction of the midgut epithelium. On the third day after infection with *Bt* . var . aizawai HD-282 Fig. (2) shows the shrinkage and separation of the midgut cells from each other leaving many vacuoles and partially shrinkage in Pritrophic membrane. On the fourth day Fig. (3) shows lyses of the epithelial cells from each other, swelling and rupture of some cells, an increase in vacuolation beside the discharge of some cells into the gut lumen. A ruptured Pritrophic membrane and basement membrane with their musculosa were also observed. On the fifth day after infection with *Bt* (Fig 4) , the Pritrophic membrane was completely destroyed, disintegration of microvilli and partial hypertrophy of the midgut cells where they were elongated and swollen (Fig.5). On the sixth day shows complete destruction of the midgut epithelial cells, increase in vacuolation, the cells shows bulbous eversion of the apical plasma membrane and musclosa is detached from the epithelium. The creaks in the cell cytoplasm may occur due to loss of its elasticity.

Generally, the distal ends of the cells (striated border) were damaged and ulcerated areas were present and the gut lumen was filled with debris resulting from the destroyed cells. Lyses of some nuclei took place, and total disintegration of the mesenteric epithelium occurred shortly afterwards before the complete disappearance of the characteristic shape of the cell (Fig. 6). The swelling of the cell is a common response in lepidopterous larvae infected with *Bt*. (Fast, 1981, Salama& Sharaby1985, Pandey *et al*. 2009). The swelling and the lysis of the intestinal cells indicate the penetration of the fluid into the cells. This phenomenon could be related to the alteration of the system of intermembranous ionic regulation (Fast and Morrison,1972; Gringorten ,2001; Luca *et al.,* 2012). The fact that changes in the gut can not only affect their development, but also cause major physiological events, such as changes in nutrient absorption, degenerative transformation, appetite loss and abandonment of food, gut paralysis, physiological disorders, and total paralysis.

E. Effect on the Integument.

The epidermis has a basement membrane that appears as an amorphous granular layer. The epidermis forms a continuous sheet of polygonal cells below the cuticle, each has numerous large nucleoli. The endocuticle which constitutes the bulk of the integument is composed of numerous lamellae. These lamellae as patterns of micro fibrils are arranged in sheets which curve out at right angels between sheets. Exocuticle is lamellated and it lies between the endocuticle and Epicuticle. The Epicuticle contains wax and cement layers that cover the entire surface of the cuticle (Fig.7). Infection with *Bt*. var. aizawai HD-282 caused clumping of both exo – and endo-cuticle with an obvious separation from each other(Fig. 8).

F. Effect on the Nerve ganglion.

The fourth abdominal nerve ganglion occupies the central region of the nerve. Nerve cells exist on the periphery of this ganglion and lie beneath the neurilemma, while the central parts are occupied by a Neuropile mass of fibrous tissues (Fig. 9). Infection with the *Bt* causes a marked degeneration of the nerve cells and vacuoles were observed. The nerve fibers as well as the nerve cells showed a vacuolated area and the neurilemma were partially destroyed (Fig. 10).

G. Effect on the fat bodies.

Normally , the fat cells are closely adherent to each other (Fig.11) and the external surfaces of the cell masses are covered by a delicate membranous sheath. The cytoplasm of the cells is homogenous free from vacuoles. As a result of infection with *Bt* var. aizawai HD-282, destruction and vacuolization of the fat body cells was observed (Fig.2, 4 and 12). The fat tissues gradually changed, to be soft and became easily crushed than the healthy ones with compact tissues (Fig.11).

H. Effect on the Malpighian tubules.

Three pairs of Malpighian tubules occur around the midgut . One lying dorsally, the other laterally and the third ventrally. The terminal end of the three Malpighian tubules enter the rectal walls of their anterior parts. The muscles surround the extreme proximal portion of the tube at its junction with the gut. Each tube is covered with basement membrane made up of few cells in one layer. Usually one cell surrounded one-half or two thirds of the lumen of the tubule. Malpighian tubule epithelial cells always show apical microvilli (Fig. 11). After four days infection , a degeneration of epithelial cells with its micro accumulation of the excretory products in the haemolymph and thus causing septicemia (Fig. 12).

I. Effect on the trachea and tracheoles.

Normally , the cells of tracheoles are narrow and long in shape. The cells has a basal lamina, is relatively smooth with only a few folds (Fig.13). Infection with *Bt*. on fourth day after treatment caused and excessive cellular hypertrophy of the tracheoles. The basal lamina surrounding the tracheoles was detached (Fig. 14) leaving a hollow structure and this may affect the process of gas exchange.

In the literature several studies were reported that throw some light on the mechanism of action of *Bt* δ- endotoxin as well as describing the histopathology following the administration of the toxin of susceptible insect species. Most of those studies were carried out on the silk worm *Bombyx mori* , Endo and Nishiitsutsuji-Uwo,(1980) cotton leaf worm *S. littoralis*, Abo-EL-Mhasen,(2016), Stink bugs *Anticarsia gemmatalis*, Schunemannet. *et.al* (2014) , western corn rootworm larvae *Diabrotica vigifera virgifera*, Andrew *et.al*, (2017.),Endo and Nishiitsutsuji-Uwo,(1980) , *Heliothis armigera*, Abd EL-Ghany *et.al* (2015) and the fruit fly *Bactrocera dorsalis*, Mona Fatin Syazwanee *et .al.* (2016). Although there some discrepancies in the findings regarding the mode of action of *Bt*. δ-endotoxin, yet there are general agreement on the bases of the δ-endotoxinaction. Thus, it is assumed that the susceptible host to a given type of *Bt* . δ-endotoxin has in the gut some proteolytic enzymes capable of breaking down the crystal into its subunits that some of which are toxic to that insect species. When the susceptible insect eats δ-endotoxin along with its diet certain histopathlogical changes take place. First, the gut become paralyzed accompanied with significant damage to the intestinal wall. These changes are followed by a toxemia and. Or septicemia taking place some times after the gut paralysis. Endo and Nishiitsutsuji-Uwo,(1980) studied the histopathlogical changes in the midgut of the silk worm *Bombyx mori*. They divided the succession of symptoms in the intact larvae into four arbitrary stages. In the first stage appearance and locomotion of the larva is normal but the animal stops feeding. The next stage starts with the sluggish movement that progresses with time trough the third stage and ending up with complete paralysis that becomes evident in the last stage. They concluded that the action of δ-endotoxin is highly specific to the midgut since contractile movement of both fore gut and hind gut could be observed for a long time after all locomotors activity and heart beat have stopped. They also noted that there is an associated

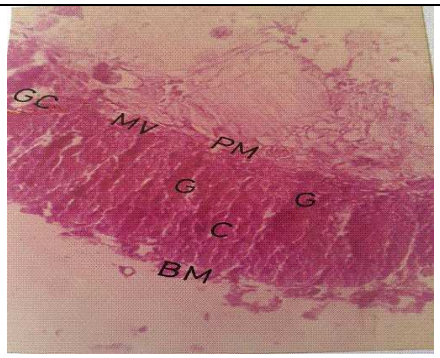

Fig:1- Cross section in normal midgut larva.X50 50

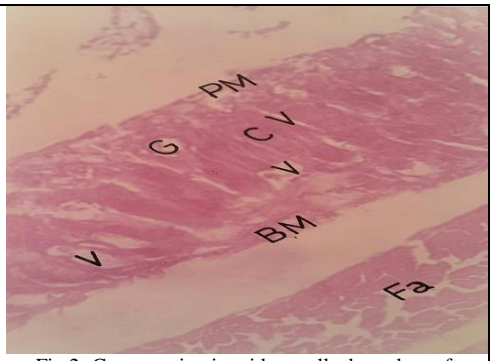

Fig.2- Cross section in midgut cells three days after infection. X 100.

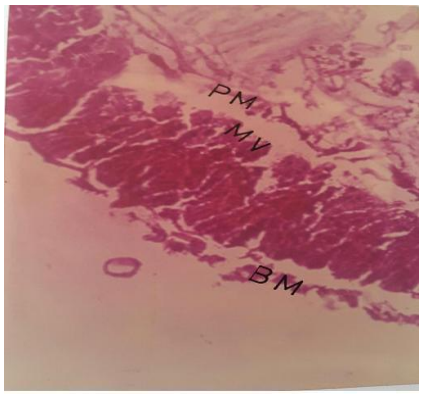

Fig:3- Cross section in midgutcellc four days after infection. X 50.

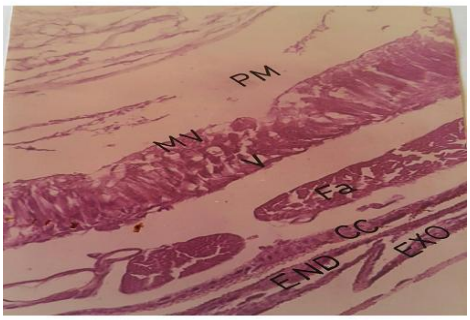

Fig:4-Cross section in midgut cells five days after infection. X 50

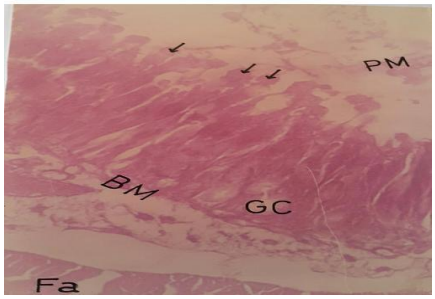

Fig:5- Cross section in midgut five days after infection showing hypertrophy of the epithelial cells making balloon shape ,(marked with black arrows. X 100.

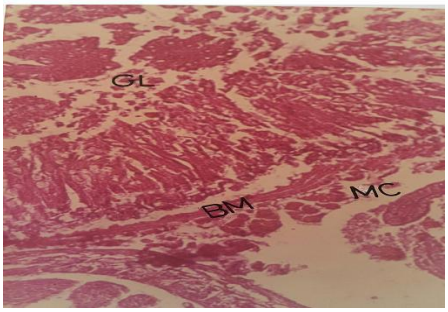

Fig: 6- Cross section in the mid gut showing complete damage of the epithelial cells . x 50

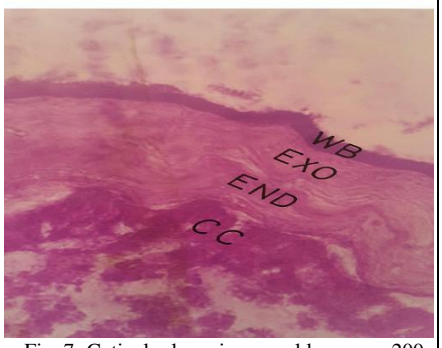

Fig :7- Cuticular layer in normal larva . x 200

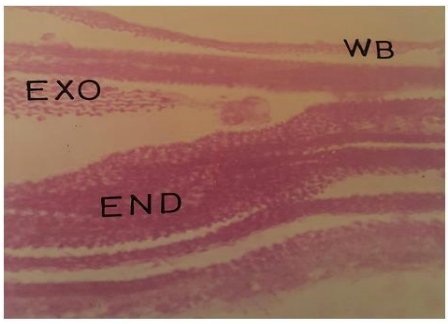

Fig:8- Cuticular layer in infected larva. X 200

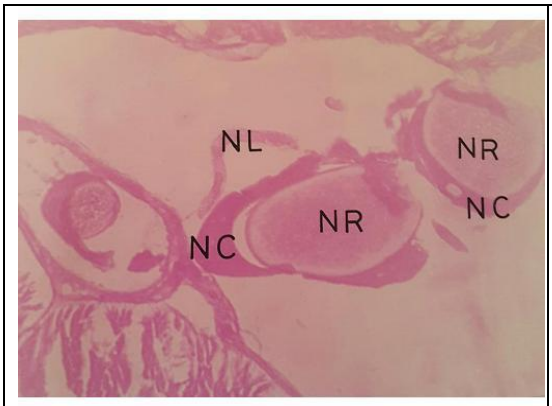

Fig:9- Cross section in normal nerve ganglion. X50

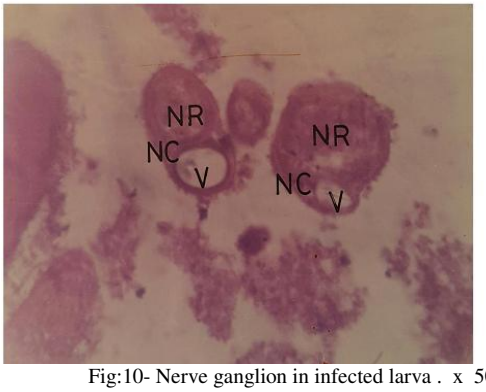

Fig:10- Nerve ganglion in infected larva . x 50

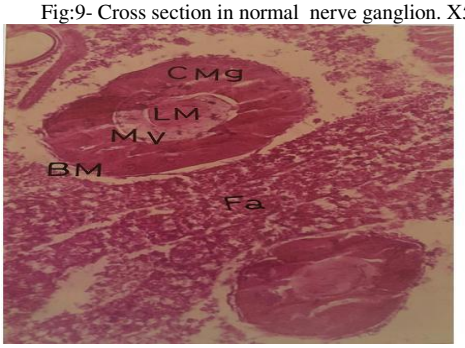

Fig: 11- Malpighian tubules in normal larva . x 50.

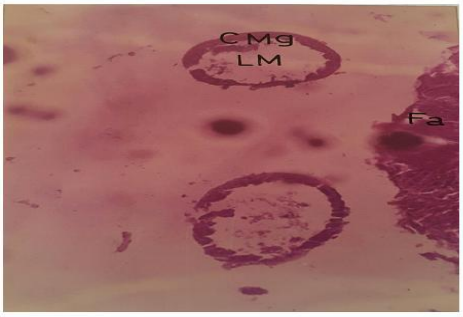

Fig:12- Malpighian tubules in the infected larva . x 50.

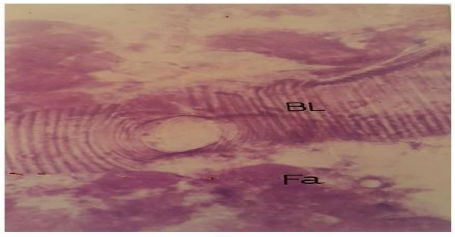

Fig:13- section through tracheoles in normal larva. X200

Fig:14- Tracheoles in infected larva. X 200

Figures abbreviation:

Goblet cavity (GC), Microvilli(MV) , Pritrophic membrane(PM), Basement membrane (BM), Goblet cells (G), Columnar cells (C), Vacuoles (V), Fat body (Fa), Exo-cuticle (EXO), Endo-cuticle (END), Circular muscles (CC), Gut lumen (GL), Masculosa layer (MC), Wax bloom (WB), Nerve cells (NC), Neurolemma (NL), Neuropile mass (NR), Malpighian tubule cells(CMG), Lumen of Malpighian tubule(LM), Tainidia or folds of tracheoles(BL).

abrupt rise in the concentration of potassium ion (k^+) in the haemolymph immediately after the silkworm stop feeding and blood PH sharply rises. Also studied the histopathlogical changes on the molecular level. They noted quit different ultra structure changes in the columnar cells and goblet cells of the midgut of *Bomoyx mori* . Shortly after the ingestion of the δ-endotoxin the deep infloding of the basal membrane of some columnar cells became very irregular in shape and the mitochondria near the basal region were transformed into a condensed appearance (Abdel-Razik *et. al.*2010, Gupta& Dikshit, 2010). Thomas & Ellae (1983) suggested that an insecticidal mechanism of *B t* in which interaction of toxin with specific plasma membrane lipids causes a detergent like rearrangement of the lipids, leading to disruption of membrane integrity and eventual cytolysis. Himeno *et al.*(1985) suggest the participation of nucleotide derivatives in the action of the δ-endotoxin. Sacchi *et al*. (1986) found that the *Bt*. toxin inhibits the uptake of amino acids by brush border membrane vesicles prepared from midgut of *Pieris brassica* larvae. They reported that the toxin increases the k^+ permeability of the membrane. Sing *et.al.*(1986) studied the toxic action of *Bt.* var israelensis in *Aedes aegypti* in vivo. They found that the skeletal muscles swell, the plasma membrane separates from underlying myofibrils, and mitochondria lose their structural integrity. They suggested that necrosis of skeletal muscles is principal cause of paralysis of *B.t.* treated insects. The rapid disruption of cellular fine structure supports a hypothesis based on an interaction of toxins with the epithelial cell membranes reminiscent of the specific *B. thuringiensis* δ-endotoxin mechanism of action on other insect targets (Luca *et.al* (2012).

CONCLUSION

It appears that the histopathlogical effects caused by feeding the larvae of *S. littoralis* on *Bt* δ-endotoxin var aizawai HD-282 are mainly localized in the midgut, cuticle, nerve ganglion, muscles surrounding the alimentary canal. and the fat body. These are the most common pathological changes observed from the moment the susceptible insects ingest the B.t spores and crystals, leading to insect death. These findings advance our understanding of the insect cell biology and pathology of these insecticidal proteins, which should

further the field of insect resistance traits and cotton leaf worm *S.littoralis* management.

REFERENCES

[1] Salama.H.S, Sharaby.A. 1985: Histopathlogical changes in Heliothes armigera infected with Bacillus thuringiensis as detected by electron microscopy. Insect.Dci.Applic. 6(4):503-511.

[2] Salama.H.S, Foda.M.S, and Sharaby. 1983: Biological activity of mixture of Bacilus thuringiensis varieties against some cotton pests. Z.angev.ent. 95: 69-74.

[3] Salama. H.S, Foda. M.S, Sharaby. A, and Salem. S. 1991: Potency of Bacillus thuringiensis Berliner strains against major pests of oil-seed crops. J.Appl. Ent, 111:418- 424.

[4] Abo-EL-Mahasen.M.M. 2016. Histopathlogical effects of Emamectin Benzoate on larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Egypt.J.Biol.Pest.Control. 26(1):147-152.

[5] Andrew. J.B, Heather. E.P, Huarong.Li, Sek.Y.T, Steven.L.E, and Kenneth. E.N. 2017. Histopathlogical effects of Bt and AcdA Insecticidal proteins on the midgut epithelium of 2017western corn rootworm larvae (*Diabrotica vigifera vigifera*) . MDPI toxins.9(5): 156-166. https://www.ncbi.nih.gov/pmc/articles/PMC5450704/

[6] Abdl EL-Ghany. N.M, Saker. M, Salama. H.S, Ragaie. M. 2015. Histopathology of larval midgut of Heliothes armigera (Hubner) fed on *Bacillus thuringiensis* crystals and Bt-tomato plants. J. Genetic Engineering and Biotechnology. 13: 221-225.

[7] Abdel-Razek. A.S, Morris. O.N, White. N.D.G, Salama. H.S. EL-Morsy. A, AND Abou-Ela. R. 2010. Comparative histopathology of *Plodia interpunctella* (Lepidoptera: Pyralidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) as affected by *Bacillus thuriniensis* varaieties *Indiana* or *Morrisoni*. Archive of Phytopathology and Plant Protection. 307-320. http://www.tandfonline.com/dio/abs/10.1080/0325400216132

[8] Dulmage. H.T, Boening. O, Rehenborg, C, and Hansen. G. 1971 . A proposed standardized bioassay for formulation of Bacillus thuriniensis based on the international unit. J. invetebr. Pathol. 18: 240-245.

[9] Endo. Y, and Nishiiitsutsuji-Uwo. J. 1980. Mode of action of *Bacillus thuringiensis* δ-endotoxin: Histopathlogical changes in the silkworm midgut. J.invertebr. pathol. 36: 90-103.

[10] Fast. P.G.1981. Bacteria- The crystal toxin of *Bacillus thuringiensis.* In: Microbial control of pests and plant diseases(H.D. Burges, ed.) pp. 223- 248. Academic press, New York.

[11] Fast. P.G., and Morrison. I.K. 1972. The δ-endotoxin of *Bscillus thuringiensis* IV. On ion regulation by midgut tissue of Bombyx mori larvae. J.Invert. Pathol. 20 :208.

[12] Griego. U.M, Moffett. D, and Spence. K.D.1979. Inhibition of active potassium ion transport in the tobacco horn worm *Manduca sexta* midgut after ingestion of *Bacillus thuriniensis* endotoxin. J. Insect Physiol.25(3): 283- 288.

[13] Gringorten. J.I. 2001. Ion balance in the lepidopteran midgut and insecticidal action of *Bacillus thuriniensis*. In biochemical sites of insecticide action and resistance edited by Ishaaya. I, Heidelberg: Springer. Pp, 167-207.

[14] Gupta. S, and Dikshit. A.K.2010. Biopesticides: An ecofrindly approach for pest control . J of Biopesticides. 3: 168-188.

(15) Heimpel. A.M, and Angus. T.A. 1959. The site of action of crytalliferous bacteria in LePidopterous larvae. J. Insect Pathol. 1: 152-170.

[15] Himeno. M, Koyama. N, Funto. T, and Komano. T. 1985. Mechanism of action of *Bacillus thuringiensis* insecticidal δ-endotoxin on insect cells in vitro. Agric. Biol. Chem. 49(5): 1461-1468.

[16] Hukuhara. T, Midorikawa.M, and Iwahana. H. 1984. The effect of δ-endotoxin of *Bt*. on the gut movements of the silk worm *Bombyx mori*. App.Entpmol.Zool. 19(2) 270-272.

[17] Luethy. P, Studer. D.1986. Control of simuliid black field and mosquitoes with *Bt*. Subsp. Israelensis. Mircen. J.Appl.Microbiol. Biotechnol, 2(1):91-99.

[18] Luca. R, Alberto. A, and Ignazio. F. 2012. Observations on house fly larvae midgut ultrastructure after *Brevibacillus laterosporus* ingestion. J. Invertebr. Pathology, 111(3): 211-216.

[19] Morris. O.N.1991. Development of *Bacillus thuringiensis* for the control of Lepidopteran pests of oilseed crops. Agricure. Canada-IDRC joint project report Ottawa, Canada.

[20] Mohsen. Z.H, AL-Chalabi. B.M, and Mehdi. N.S. 1967. Midgut histopathology in culex *quinque fasciatus* say treated with *Bt*. H-14 and *B sphaericus*. J.Biol.Sci.Res, 18(1): 219-228.

[21] Mona Fatin Syazwanee. M.G, Noormasshela. U.A, Nor Azwady. A.A, Rusea. G, and Muskhazli.M. 2016. *Bacillus thuringiensis* entomotoxicity in wastewater sludge-Culture medium towards Bactrocera dorsalis and their hestopathological assessment. Sains Malaysiana.45(4) :589-594.

[22] Nishiitsutsuji-Uwo. J, and Endo. Y. 1980. Mode of action of *Bacillus thuringiensis* δ-endotoxin. General characteristics of intoxicated *Bombyx mori* larvae. J. Inverteb. Pathol. 35 :219-224.

[23] Pandey. S, Joshi. B.D, and Tiwari. L.D.2009. Histopathlogical changes in the midgut of *Spodoptera littura* larvae on ingestion of *Bacillus thuringiensis* delta-endotoxin . Archives of Phytopathol and Plant Protection. 42: 376-383.

[24] Sacchi. V.f, Parenti. P, Hanozet. G.N, Giordana. B, Luethy. P, and Wolfersberger. M.G.1986. *Bacillus thuringiensis* toxin inhibits K^+ gradient-dependent amino acid transport across the brush border membrane of *Pieris brassica* midgut cells. FEBS Lett. 204(2) : 213-218.

[25] Schunemann. R, Kann, N, and Fiuza. L.M. 2014. Mode of action and specificity of *Bacillus thuringiensis* toxins in the control of caterpillar and stink bugs in soybean culture. ISRN Microbiology. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3918364.

[26] Sareen. V, Rathore. Y.S, and Bhattacharya. A.K. 1983. Influence of *Bt* . var *thuringiensis* on the food utilization of *Spodoptera littura*. Z. Angew. Entomol. 95(3) : 153-258.

[27] Singh. G.J.P, Schouest. J.R, and Gill. S.S. 1986. The toxic action of *Bt*. Var. *israelensis* in *Aedes aegypti* invivo. II. The relevance of skeletal muscle system lesion to its poisoning syndrome. Festic. Biochem. Physiol. 26(1): 47-55.

[28] Thomas. W.E, and Ellar. D.J.1983. Mechanism of action of *Bacillus thuringiensis* var. *israelensis* insecticidal δ-endotoxin. FEBS.Lett. 154(2) : 362.