The Efficacy of Different *Bacillus Thuringiensis* Formulations for the Control of the Cotton Leafworm *Spodoptera Littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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Abstract: The efficacy of three *Bacillus thuringiensis* formulations, Agerin, Dipel 2X and Dipel DF were tested against 2nd larval instar of *Spodoptera littoralis*. The three formulations were tested in the laboratory, field and semi field experiments. The 48 hour LC_{50} for Agerin, Dipel 2X and Dipel DF were 0.18, 0.07 and 0.10 % for the three formulations, respectively. The results of the field experiment indicated that the general mean of reduction were 59.0, 55.9 and 58.6 % for the three *Bt* formulations (Agerin, Dipel 2Xand Dipel DF, respectively). In addition, the general mean of mortality rate in the semi-field experiments were 60.3, 60.4 and 61.3 % for Agerin, Dipel 2X and Dipel DF, respectively. Moreover, the histopathological studies using ultrastructure microscopy were carried out on the midgut of 4th larval instar after the treatment of the second instars with LC_{50} of the three formulations. These results therefore confirm the opinion stated that the toxicities of the different three formulations, are similar to each other. Therefore the Egyptian *Bacillus thuringiensis* strain (Agerin) can be used for control of *S. littoralis* as it is cheap and readily available.

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1. Introduction:

The cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is a serious polyphagous pest that damages numerous kinds of cultivated crops including corn, cotton, beet, tomato, and many others. Due to the overuse of insecticides over the past years, *S. littoralis* has developed resistance to various synthetic insecticides. Therefore alternative bioinsecticides need to be evaluated (Fahmy and Dahi 2009). *Bacillus thuringiensis* strains are high toxic to *S. littoralis* (Sneh *et al.,* 1981 and Yunovitz *et al.,* 1986). *Bacillus thuringiensis* (*Bt*) is the most widely applied biological insecticide and is used to manage insects that affect forestry and agriculture and transmit human and animal pathogens (Broderick *et al.,* 2006). *It* is a common gram-positive aerobic entomopathogenic endospore forming soil bacterium, which produces unique crystalline cytoplasmic inclusion bodies during the process of sporulation (Martin and Travers 1989). These crystals are predominantly comprised of one or more cry proteins, also called δ-endotoxins. These toxins are highly specific to their target insect, are harmless to humans, vertebrates and plants, and are completely biodegradable (Bravo *et al.,* 2005).

The knowledge of toxicity of different commercial formulations of *Bt* strains to the *S. littoralis* is important for their optimal use in the form of spray formulation or in the development of *Bt* transgenic cole crops. The present study, therefore, reports toxicity of three commercial formulations of *Bt* strains, Agerin (Egyptian commercial formulation), Dipel 2X and Dipel DF, against 2nd larval instar of *S. littoralis*. Furthermore, histopathological studies using ultrastructure microscopy also will be carried out on the midgut of 4th larval instar after the treatment of the second instars with LC_{50} of *Bt* formulations.

2.Material and Methods:-

Rearing of *Spodoptera littoralis* in laboratory:-

The colony of cotton leafworm *S. littoralis* was obtained from the division of the cotton leafworm, Plant Protection Resarch Institute, Dokki, Egypt. Larval stages were reared on castor bean leaves at 27±2°C and 65±5% R.H. as described by El-Dafrawi *et al.,* (1964).

Bacillus thuringiensis formulations:-

Three *Bacillus thuringiensis* formulations, wettable powder, used in the present study are Agerin 6.5%, Dipel 2X 6.4%, and Dipel DF 54%, containing 32000 international units per mg. (IU/mg) of product. Agerin *(Bacillus thuringiensis aegypti)* a product of Agricultural Genetic Engineering Research Institute (AGERI). Dipel 2X and Dipel DF (*Bacillus thuringiensis*, subsp. *Kurstaki*), supplied from May trade Corporation.

Bioassays

Leaf-dip bioassay method was followed as described by Tabashnik *et al.,* (1993) using castor leaves. The leaves were first washed with distilled water and dipped in solutions of different concentrations of *Bt* formulations which prepared with distilled water. Each leaf was dipped for 5–10 Sec and allowed to air dry for a period of 1 hr. Then the leaves were placed individually into Petri dishes (15 cm dia.). Newly hatched second instar larvae were released on each dish with three replications (twenty insects /replicate) including controls. Different concentrations of Agerin, Dipel 2X and Dipel DF were prepared in distilled water. The concentrations of Dipel 2X and Agerin were 0.325%, 0.1625%, 0.08125%, 0.04063%, 0.0203% and 0.0102%. The concentrations of Dipel DF were 2.7%, 1.35%, 0.675%, 0.3375%, 0.169% and 0.0845%. Larvae were allowed to feed for 48 hrs. Larval mortality was recorded at 48 hrs. Larvae that treated with LC_{50} of the three formulations were fed on untreated leaves after 48 hrs of treatments and followed until adult emergence to assess pupal and adult malformations.

Field experiments:

The experiments were conducted at Toukh District, Qalyobia Governorate to evaluate the field efficiency of three *Bacillus thuringiensis* formulations (Agerin 6.5 % WP, Dipel 2x 6.4%, WP and Dipel DF54 % WP) against cotton leafworm *Spodoptera littoralis*. The field area was cultivated with Giza 86 cotton variety and the normal agricultural practices were applied. The experimental area was divided into plates of 1/16 feddan (262.5 m2) one for each *Bt* formulation. The treatments were arranged in Randomized Complete Blocks Design (RCBD) with four replicates per each *Bt* formulation. Application with the three *Bt* formulations were on July 2009. A motor sprayer was used. The volume of spray solution was 100 liters / Feddan. The number of *S. littoralis* larvae were recorded before the spray and on 2, 4, 6 and 8 days after the spray on one meter lengthwise cotton plants for five times (four at corners and the last one on plot center) for each *Bt* formulation. The reduction % in the *S. littoralis* larvae population was estimated by using technique of Henderson and Tilton (1955).

Semi Field experiments:

From the same experiment area, the treated cotton leaves with the three *Bt* formulations were collected after zero time, 1, 2, 3, 4, 5, 6 and 7 days and directly transfer to the laboratory for feeding the second instar larvae of *S. littoralis* to estimate the general mean of mortality percentages for the three *Bt* formulations.

Statistical Analysis:-

The percentage of mortality was corrected according to the Abbott formula (Abbott 1925) for correction wherever required. Probit analysis was determined to calculate $LC_{50,}$ Finney (1971), through software computer program. Statistical significant differences between individual means were determined by one way analysis of variance (ANOVA).

Histopathological studies:-

After the treatment of the 1-day old 2nd instar larvae of *S. littoralis* with LC_{50} of three tested *Bt* formulations for 48 hrs, the histopathological and ultrastuctural effects were examined in the mid-gut of the 1- day old 4th instar . For this purpose, a representative larvae of each group were fixed as soon as possible in 3% phosphate buffered glutaraldehyde (pH 7.3) for 2 hours. After two rinses in the buffer (for a period of 4 hours) the specimens are post fixed in 1% buffered osmium tetroxide for 1 hour at 4°C (Brissan *et al.,* 1996). The specimens were washed twice in a buffer for 30 minutes. The specimens are then dehydrated in the seconding grades of ethanol, 50, 70, 80, 90 and 100%. The specimens were cleared in toluene for 10 minutes and then embedded in the resin of choic Epon. Semi-then sections are cut from these blocks (stained with toluidine blue) and examined by the light microscope (Spnrr 1969). Ultrathin sections obtained from selected blocks were mounted on copper grids stained with uranyl acetate and lead citrate and then examined with Jol 1010 transmission electron microscope (Reynolds 1963). This technique was carried out at Faculty of Science, Ain Shams University, Cairo, Egypt.2. Materials and Methods:

This was a retrospective study. The clinical courses of 47 recipients who were transplanted for HCV end stage liver disease and successfully survived a least 6 months post LDLT. The Immunosuppression regimens for all our patients included corticosteroids which were all tapered within the first 3 month. Calcineurin inhibitors used were either tacrolimus (FK) or ciclosporine (Neoral). Mycophenolate mofetil was given to all except 4 patients who had monotherapy with tacrolimus as they had hepatocellular carcinoma before transplantation. Hepatitis C virus recurrence was suspected in cases of elevated liver enzymes with a usual high level of viraemia and then all subjected to a liver biopsy and proved histologically by our transplant pathologist. The studied group was divided into two groups; group I had basiliximab as induction therapy and group II had no basiliximab as induction

therapy and the incidence of recurrence was compared between the two groups.

3. Results:

Field experiments:

The data presented in Table (1) showed that the reduction rates in *S. littoralis* larvae population in the field after the treatment with the recommended doses of the three *Bt* formulations. The reduction rates were 23.2, 51.4, 77.7 and 86.0 % after 2, 4, 6 and 8 days from treatment, respectively for the 1st formulation (Agerin). The reduction rates for the 2nd formulations (Dipel 2X) were 24.8, 43.9, 68.0 and 86.0 % after 2, 4, 6 and 8 days from treatments, respectively. For the 3rd formulation (Dipel DF) it reaches 30.1, 49.7, 70.7 and 83.9 % after the same periods, respectively. The general mean of reduction rate were 59.0, 55.9 and 58.6 % for Agerin, Dipel 2X and Dipel DF, respectively.

Table (1): Reduction % of *S. littoralis* larval population after treated with recommended dose of three *Bt* formulations during cotton season 2009.

Bt commercial name	Rate of application gm / Fed.	Reduction %				General mean
		2 Days	4 days	6 days	8 days	
Agerin	250	23.2	51.4	77.7	86.0	59.6
Dipel 2x	200	24.8	43.9	68.0	86.7	55.9
Dipel DF	200	30.1	49.7	70.7	83.9	58.6

Semi Field experiments:

Data in Table (2) shows the corrected mortality percentage for *S. littoralis* 2nd instar larvae after treated the cotton plants with recommended dose of three *Bt* formulations in semi-field experiment. The corrected mortality percentage reached to the highest rate after zero time treatment for all *Bt* formulations and it reached to the lowest one after 7 days treatment. The general mean of mortality % were 60.3, 60.4 and 61.3 % for the three *Bt* formulations (Agerin, Dipel 2X and Dipel DF, respectively).

Table (2): Corrected Mortality % of *S. littoralis* larvae in the field after the treatment with recommended dose of three *Bt* formulations during cotton season 2009.

Bt Commercial name	Rate of application gm / Fed.	Corrected mortality %								General mean
		Zero time	1 day	2 days	3 days	4 days	5 days	6 days	7 days	
Agerin	250	70.5	69.8	69.1	60.6	57.9	57.7	50.0	46.8	60.3
Dipel 2X	200	72.3	71.7	68.8	66.0	63.8	56.3	44.9	39.6	60.4
Dipel DF	200	72.0	68.1	67.7	64.1	62.1	58.9	52.1	45.2	61.3

Bioassays

Table (3) shows that LC_{50} estimated values for Agerin, Diple2X and DipleDF were 0.18, 0.07 and 0.10 % for the three formulations, respectively.

Table (3): Toxicity of commercial formulations of *Bacillus thuringiensis* to *S. littoralis* after 48 h of exposure.

Bt commercial formulations	LC_{50} %
Agerin	0.18
Dipel 2X	0.07
Dipel DF	0.10

Metamorphic and morphogenic effects:

The morphogenic abnormalities of pupae and adults which emerged from 2nd instar larvae which treated with LC_{50} of Diple DF, Diple 2X and Agerin (Fig. 2,3&4, respectively) could be grouped into four categories malformed larvae, malformed larval-pupal intermediates, malformed pupae and malformed adults as compared with normal larva, pupa and adult stages (Fig. 1). Diple 2X induced swallowed larvae with a ring of larval cuticle around the tip of abdomen (Fig. 3-A). Malformed larval-pupal intermediates that produced by the action of Diple DF, Diple 2X and Agerin can be easily observed in Fig. (2-A&B), Fig. (3-B) and Fig. (4-B), respectively. Pupae with small size were observed in case of Agerin (Fig. 4-A right) with compared to control (Fig. 4-A left). Some emerged adults have various degrees of morphogenic abnormalities. Adults were unable to emerge from their pupal skins (Fig. 2-C). Adults were completely free but possessed crumpled and incomplete formation of wings (Fig. 2-D & 3-C). Collapsed appendages and evaginated elytra (4-C).

Histopathological studies:

The histopathological and ultrastuctural investigations were carried out for 1- day old 4th instar larvae after feeding the 1- day old 2nd instar on castor leaves treated with LC_{50} of Agerin, Diple2X or Diple DF for 48 hours.

Ultrathin sections of the non-infected *S. littoralis* control mid-gut showed that the apical folded plasma membrane ,microvilli, (Mv) of epithelial cells are arranged in a regular array (Fig. 5-A). The coated vesicles (Cv) lie in the cytoplasm immediately adjacent to the base of the microvilli accumulates along the apical region of the cells. The endoplasmic reticulum is represented in fairly well developed rough form (RER) as long and short rod-like cisternae. Free ribosomes are found in the cytoplasm of the columnar cells (Fig. 5-A&B). The mitochondria (Mi) are distributed throughout the cytoplasm of the midgut cells. They are rounded or elongated in shapes and have various sizes (Fig. 5-B).

When the mid-gut of larvae was exposed to Diple DF, it showed increasing in the morphological changes of the epithelium Fig. (6-A). Microvilli of the pithelium were irregular and broken into the lumen in the form of blebs. Irregularities in mitochondrial structure could be observed. Disarray of cristae with more or less disintegration was also observed. In addition various sizes of round granules appear in the cytoplasm. They give rise to large vacuoles (Va) which may be liberated separately into the gut lumen through the striated border or they may first coalesce into a single large vacuole. The nuclear membrane (Nm) showed irregular shape (Fig. 6-B). The nuclear chromatins (Ch) appear distributed in large and small clumps. It was released by the rupture of nuclear envelop. The cytoplasm around the nucleus filled with *Bt* cells.

The apical part of the epithelial cell showed various degrees of damage after treatment with Agerin. Microvilli appeared activated with irregular, curled and dense shape. It filled with *Bt* cells (Fig. 7-A). Many vacuoles were observed in the apical part of the epithelial cell. Vacuoles appear to slough from microvilli into the lumen. Some of mitochondria degenerated whereas other ones maintain its morphology. Also multi-vesicular bodies (MVB) appeared in the cell wall which were transported outside the cytoplasm (Fig.7-B)

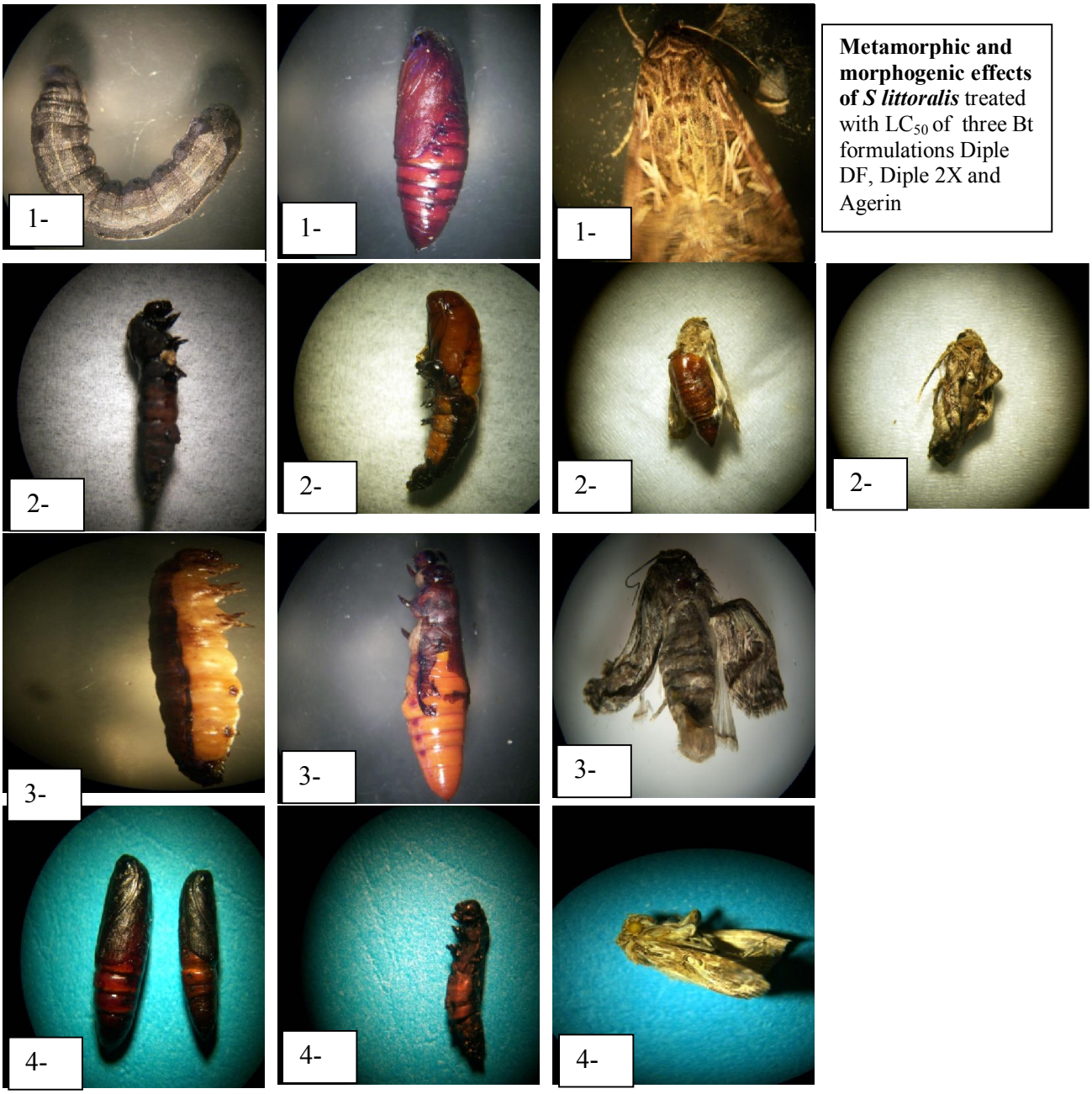
Irregular microvilli filled with bacteria and broken into the lumen was observed in the midgut of larvae that treated with Diple 2X (Fig. 8-A). Vacuolated cytoplasm, irregular shape of mitochondria with loss of cristia and apical cell membrane broken in some parts was also observed in the same figure. (Fig. 8-B). The *Bt* aggregated in large vacuole in different stages. Small vacuoles found around the rough endoplasmic reticulum was also observed.

4. Discussion:

Efficacy of the three *Bt* formulations against *S. littoralis* larvae in the field and semi-field experiments showed that the general mean of reduction rate in the field were 59.0, 55.9 and 58.6 % for Agerin, Dipel 2X and Dipel DF, respectively. While the general mean of mortality % in semi-field experiments were 60.3, 60.4 and 61.3 % for the three *Bt* formulations (Agerin, Dipel 2X and Dipel DF, respectively). It was obvious from the field and semi field experiments that no difference in the reduction rates in *S. littoralis* larvae population treated with the recommended dose of three *Bt* formulations at all sampling days. Also the bioassay data indicated that the toxicity of the three *Bt* formulations were not different from each. The LC_{50} were close together.

Morphological deformation assay was used to evaluate the toxic effect of *Bacillus thuringiensis* Cry1 Toxins (Cerstiaens *et al.,* 2001). Zd'a´rek *et al.,* (1979) studied morphogenic effects of drugs, venoms, and other neurotoxic compounds on fleshfly pupae. These authors found that agents that paralyze neuromuscular systems at the peripheral level or suppress or modify basic motor patterns centrally cause the retention of larval morphologic characters in the pupae. Early study by Peter *et al.,* (1987) indicate that *Bt* δ-endotoxin induced hyperexcited activity in the insect nervous system.

Ultrastructural observations of the midgut exposed to Agerin, Dipel 2X and Dipel DF showed similar results. Microvilli were irregular and broken, mitochondria degenerated, various sizes of round granules found in the cytoplasm and distribution of nuclear chromatins in large and small clumps. The cytoplasm filled with various size of vacuoles and *Bt* cells. many other works that report the cellular changes produced in the midgut of larvae intoxicated with the Cry proteins of *Bt* such as: an increase in the volume of the epithelium cells, rupture of microvilosities, vacuolisation of the cytoplasm, changes in the organelles of the cytoplasm and rupture of cell membrane (Mathavan *et al.,* 1989; Bauer and Pankratz 1992; Bravo *et al.,* 1992; Cavados *et al.,* 2004; Knaak and Fiuza 2005 and Knaak *et al.,* 2010). Percy and Fast (1983) illustrated that the first cell damages due to the *Bt* endotoxin in midgut was related to brush border microvilli degeneration. The mode of action of Cry toxins has been characterized principally in lepidopteran insects.



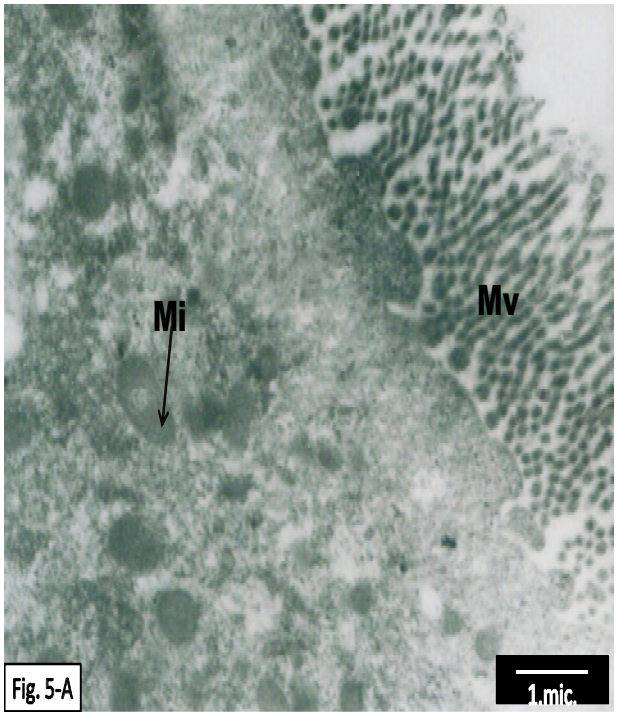
Metamorphic and morphogenic effects of *S littoralis* treated with LC_{50} of three Bt formulations Diple DF, Diple 2X and Agerin

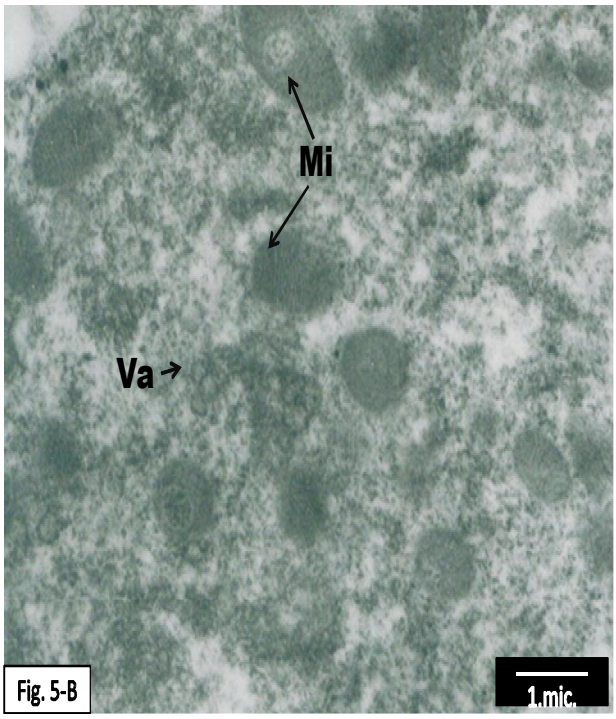
Figure: 1 (control)

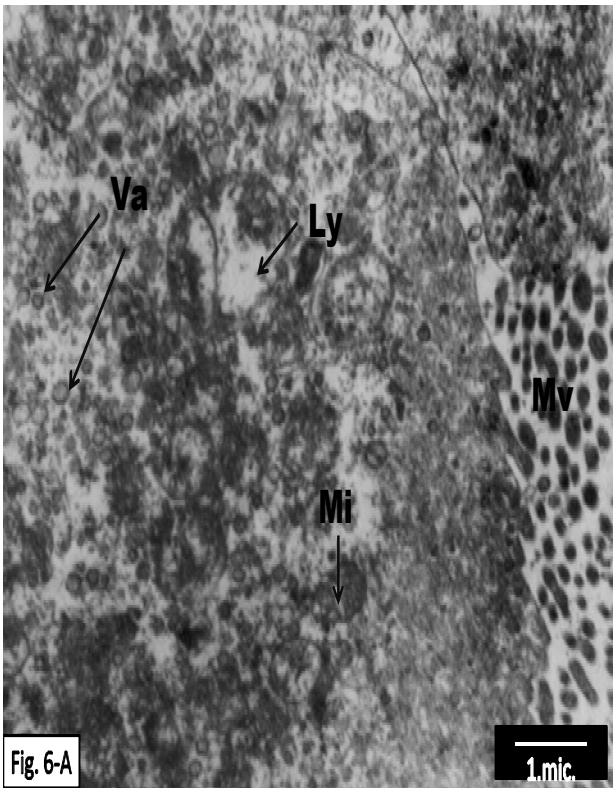
Figure: 2 (Diple Df)

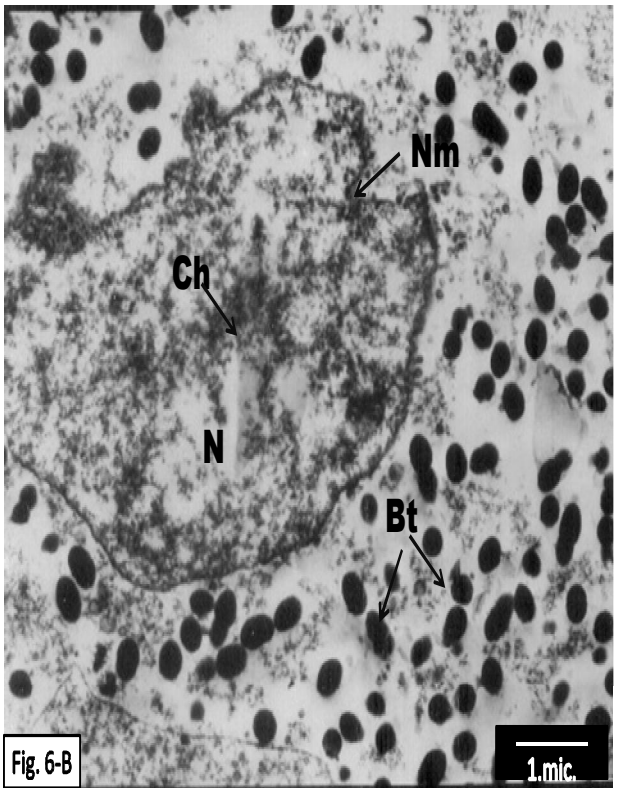
Figure: 3 (Diple

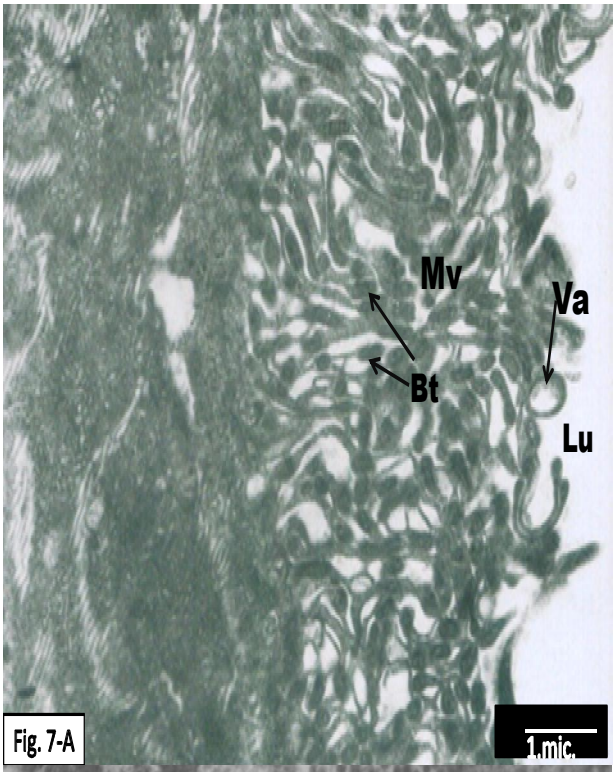
Figure: 4 (Agerin)

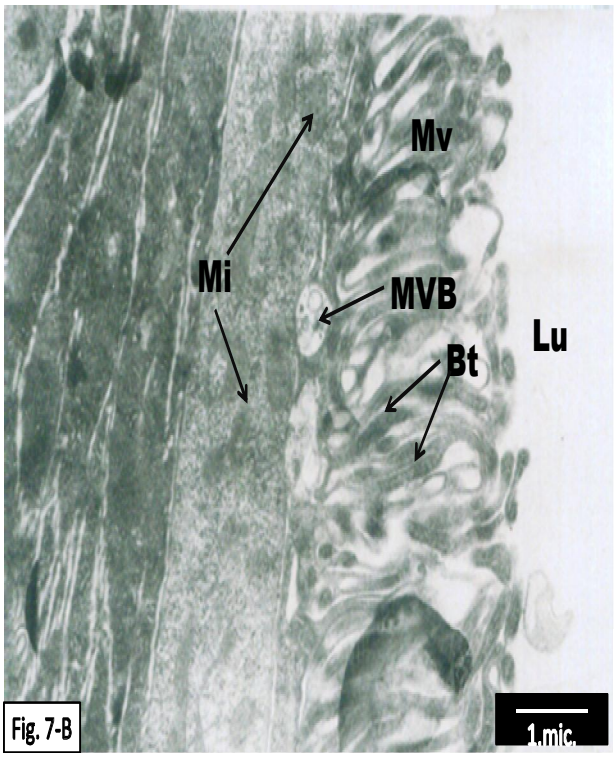

Mi
Mv
Fig. 5-A
1 mic.

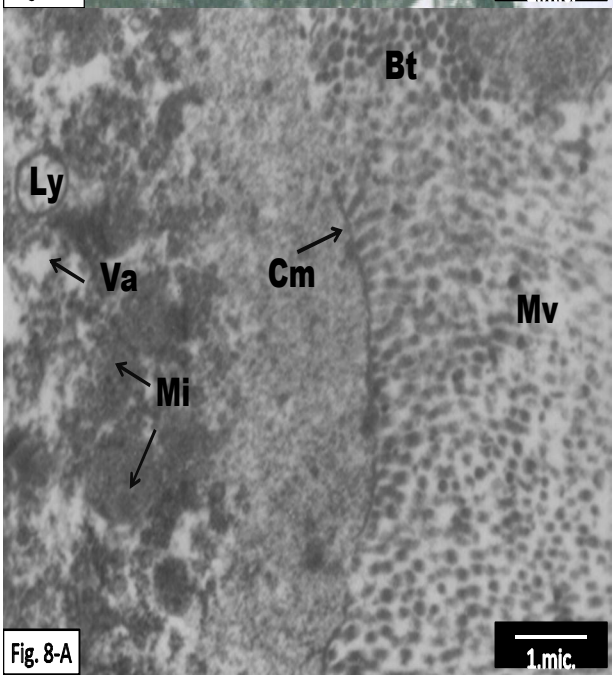

Mi
Va
Fig. 5-B
1 mic.


Va
Ly
Mv
Mi
Fig. 6-A
1 mic.


Nm
Ch
N
Bt
Fig. 6-B
1 mic.







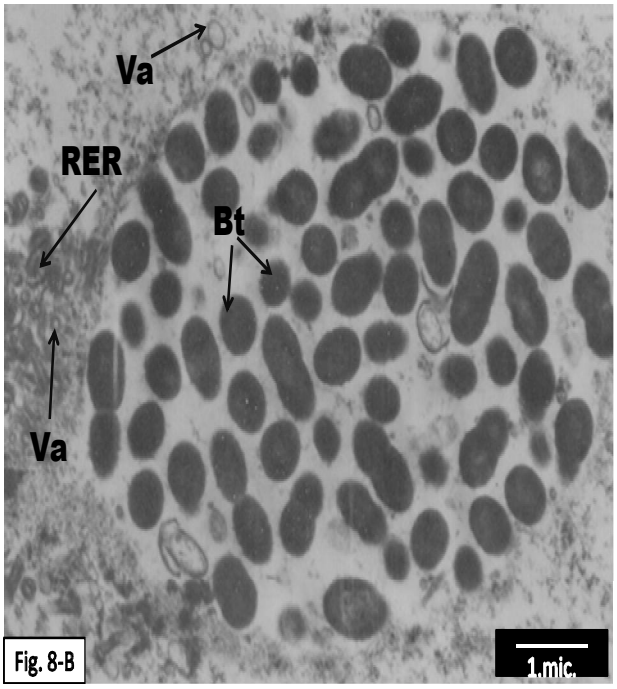


Fig. (5 A&B) ultrastuctural of the midgut for untreated 1- day old 4th instar *S. littoralis* larvae.
Fig. (6 A&B), (7 A&B) and (8A&B) 1- day old 4th instar larvae after feeding the 1- day old 2nd instar on castor

It is widely accepted that the primary action of Cry toxins is to lyse midgut epithelial cells in the target insect by forming pores in the apical microvilli membrane of the cells (Aronson and Shai 2001; de Maagd *et al.,* 2001 and Bravo *et al.,* 2005). The crystal inclusions ingested by susceptible larvae dissolve in the alkaline environment of the gut, and the solubilized inactive protoxins are cleaved by

midgut proteases yielding 60–70 kDa protease resistant proteins. The activated toxin then binds to specific receptors on the brush border membrane of the midgut epithelium columnar cells before inserting into the membrane Toxin insertion leads to the formation of lytic pores in microvilli of apical membranes (Schwartz et al., 1993). These non-specific pores render the apical membrane permeable to potassium ions as well as small molecules such as sucrose, thus disrupting the ion balance and the potential difference across the apical membrane. Selective permeability of the membrane is lost. There is significant water uptake, resulting in the cell bloating up and becoming unable to regulate osmotic pressure. There is disruption of the microvillar structures and cell organelles such as nuclei, mitochondria and ribosomes. The endoplasmic reticulum is vesciculated. The epithelial cells subsequentlyburst open in a process termed colloid osmotic lysis, (Hill and Pinnock 1998). Subsequently cell lysis and disruption of the midgut epithelium releases the cell contents providing spores a germinating medium leading to a severe septicemia and insect death (Bravo *et al.,* 2005). Injuries to mitochondria were important effects associated with all treatment. Dysfunctions of these organelles can be critical since they lead to an increase of reactive oxygen species that cause damage to major cellular components (Andreassen *et al.,* 2000). Such changes in mitochondrial morphology have previously been reported for the action of *Bt* toxins on midgut epithelia of mosquitoes and lepidopteran larvae (Lüthy and Wolfersberger, 2000; Cavados *et al.,* 2004 and Romão *et al.,* 2006).

The use of *B. thuringiensis* endotoxins originated vacuolization of the midgut epithelial cells in the different experimental models (Rey *et al.,* 1998, Cavados *et al.,* 2004). The presence of small vesicles in the cytoplasm around the endoplasmic reticulum, probably derived from the endoplasmic reticulum rupture that had lost their ribosomes (de Melo *et al.,* 2009).

These results therefore confirm that the toxicities of the different three formulations, Agerin, Dipel 2X and Dipel DF, against *S. littoralis* are similar to each other. Therefore Egyptian *Bt* strain (Agerin) can be used for control of *S. littoralis* as it is cheap and readily available.

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References

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 18: 265-267.

Andreassen, O.A.; Ferrante, R.J.; Klivenyi, P.; Klein, A. M.; Shinobu, L.A.; Epstein, C. J. and Beal. M.F. (2000). Partial deficiency of manganese superoxide dismutase exacerbates a transgenic mouse model of amyotrophic lateral sclerosis. Annals of Neurology. 47:447-455

Aronson, A.I.; Shai, Y. (2001). Why *Bacillus thuringiensis* insecticidal toxins are so effective: unique features of their mode of action. FEMS Microbiology Letters. 195:1–8.

Bauer, L.S. and Pankratz, H. (1992). Ultrastructural effects of *Bacillus thuringiensis* var. *san diego* on midgut cells of the cottonwood leaf beetle. Journal of Invertebrate Pathology. 60: 15–25.

Bravo, A.; Hendrickx, K.; Jansens, S. and Peferoen, M. (1992). Immunocytochemical analysis of specific binding of *Bt* insecticidal crystal proteins to lepidopteran and coleopteran midgut membranes. Journal of Invertebrate Pathology. 60(3): 247-253.

Bravo, A.; Gill, S.S. and Soberón, M. (2005). *Bacillus thuringiensis* Mechanisms and Use; Comprehensive Molecular Insect Science. 175-206.

Brissan, A., Gharibian, S.; Agen, R., Leclerc, D.F and Breuil, C. (1996). Localization and characterization of the melanin granules produced by the sap-staining fungus *Ophiostoma piceae*. Material and Organismen. 30: 23–32.

Broderick, N.A.; Raffa K.F. and Handelsman, J. (2006). "Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity". Proceedings of the National Academy of Sciences. U.S.A. 103 (41): 15196–9.

Cavados, C.F.; Majerowicz, S.; Chaves, J.Q.; Araujo-Coutinho, C.J. and Rabinovitch, L. (2004). Histopathological and ultrastructural effects of delta-endotoxins of *Bt* serovar *israelensis* in the midgut of *Simulium pertinax* larvae (Diptera, Simuliidae). Mem. Inst. Oswaldo Cruz. 99:493-498.

Cerstiaens, A.; Verleyen, P.; Rie, J.V.; Kerkhove, E.V.; Schwartz, J.; Laprade, R.; Loof, A. and Schoofs, L. (2001). Effect of *Bacillus thuringiensis* Cryl Toxins in Insect Hemolymph and Their Neurotoxicity in Brain Cells of *Lymantria dispar*. Applied and Environmental Microbiology. Sept.:3923–3927.

de Maagd, R.A.; Bravo, A.; Berry, C.; Crickmore, N. and Schnepf, H.E. (2001). Structure, diversity, and evolution of protein toxins from spore-forming entomopathogenic bacteria. Annual Review of Genetics. 37: 409–433.

de Melo, J.V.; Jones , G.W.; Berry, C.; Vasconcelos, R.H.T.; de Oliveira, C.M.F.; Furtado, A.F.; Peixoto, C.A. and Silva-Filha, M.H. (2009). Cytopathological Effects of *Bacillus sphaericus* Cry48Aa/Cry49Aa Toxin on Binary Toxin-Susceptible and –Resistant

Culex quinquefasciatus Larvae_ Applied and Environmental Microbiology. July : 4782–4789.

El-Dafrawi, M.E; Toppozada, A.; Mansour, M. and Zaid, M. (1964). Toxicological studies on the Egyptian cotton leafworm *Prodenia litura*, L. susceptibility of different larval instars of *Prodenia* insecticides. Journal of Economic Entomology. 57: 591-593.

Fahmy, N.M. and Dahi, H.F. (2009). Changes in detoxifying enzymes and carbohydrate metabolism associated with spinetoram in two field-collected strains of *Spodoptera littoralis* (Biosd.) Egyptian Academic journal of biological science, F. Toxicology & pest control. 1 (1): 15 – 26.

Finney, D.J. (1971). Probit analysis. Cambridge univ., London pp 333.

Henderson, C.S. and Tilton, E.W. (1955). Tests with acaricides against the brown wheat mite. Journal of Economic Entomology. 48: 157-161.

Hill, C.A. and Pinnock, D.E. (1998). Histopathological Effects of *BT* on the alimentary canal of the sheep louse, Bovicola ovis Journal of Invertebrate Pathology. 72: 9–20.

Knaak, N. and Fiuza, l.M. (2005). histopathology of *anticarsia gemmatalis* hübner (lepidoptera; noctuidae) Treated with *nucleopolyhedrovirus* and *Bt* Serovar *kurstaki.* Brazilian journal of microbiology. 36:196-200.

Knaak, N.; Franz, A.R.; Santos, G.F. and Fiuza, L.M. (2010). Histopathology and the lethal effect of Cry proteins and strains of *Bacillus thuringiensis* Berliner in *Spodoptera frugiperda* J.E. Smith Caterpillars (Lepidoptera, Noctuidae). Brazilian journal of microbiology. 70(3) : 677-684.

Lüthy, P., and Wolfersberger, M.G. (2000). Pathogenesis of *Bacillus thuringiensis* toxins, p. 167-180. *In* J. F. Charles, A. Delécluse, and C. Nielsen-Leroux (ed.), Entomopathogenic bacteria: from laboratory to field application. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Martin, P.A.W.; and Travers, T.S. (1989). Worldwide abundance and distribution of *BT* isolates. Applied Environmental Microbiology. 55, 2437–2442.

Mathavan, S.; Sudha, P.M. and Pechimuthu, S.M. (1989). Effect of *Bacillus thuringiensis* on the midgut cells of *Bombyx mori* larvae: A histopathological and histochemical study. Journal of Invertebrate Pathology. 53(2): 217-227.

Percy, J. and Fast, P.G. (1983). *Bacillus thuringiensis* crystal toxin: ul-trastructural studies of its effect on silkworm midgut cells. Journal of Invertebrate Pathology. 41: 86-98.

Peter, Y.K.C.; Dan B.; Bruce, D.H.; Michael, R. and Alford, A.R. (1987). *Bacillus thuringiensis* var. *israelensis* δ-endotoxin: Evidence of neurotoxic action. Pesticide Biochemistry and Physiology. 27(1): 42-49.

Rey, D.; Long, A., Pautou, M.P. and Meyran, J.C. (1998). Comparative histopathology of some Diptera and Crustacea of aquatic alpine ecosystems, after treatment with *Bacillus thuringiensis* var. *israelensis.* Entomol Exper Applicata. 88: 255-263.

Reynolds, S.E. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. Journal of Cell Biology. 1:208.

Romão, T.P.; Chalegre, K.D.; Key, S.; Ayres, C.F.; de Oliveira, C.M.; de-Melo-Neto, O.P. and Silva-Filha, M.H. (2006). A second independent resistance mechanism to *Bacillus sphaericus* binary toxin targets its alpha-glucosidase receptor in *Culex quinquefasciatus*. FEBS journal. 273:1556-1568.

Schwartz, J.L.; Garneau, L.; Masson, L.; Brousseau, R. and Rousseaeu, E. (1993). Journal of Membrane Biology. 132: 53–62.

Sneh, B.; Schuater, S. and Broza, M. (1981): Insecticidal activity of *Bacillus thuringiensis* strains against the Egyptian cotton leaf worm *Spodoptera littoralis* (Lep: Noctuidae). Entomophaga. 26:179-190.

Spnrr, A.R. (1969). A low viscosity epoxy resin embdding medium for electron microscopy. Journal of Ultrastructural Research. 26: 31–43

Tabashnik, B.E.; Finson, N.; Chilcutt, C.F.; Cushing, N.L. and Johnson, M.W. (1993). Increasing efficacy of bioassays: Evaluating resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). Journal of Economic Entomology. 86: 635–644.

Yunovitz, H.; Sneh, B.; Schuster, S.; Oron, U.; Broza, M. and Yawetz, A. (1986): A new sensitive method for determining the toxicity of a highly purified fraction from δ-endotoxin produced by *Bacillus thuringiensis* var. *entomocidus* on isolated larvae midgut of *Spodoptera littoralis* (Lepidoptera: Noctuidae). Journal of Invertebrate Pathology. 48: 223-231.

Zd'a´rek, J.; Slama, K. and Fraenkel, G. (1979). Changes in internal pressure during puparium formation in flies. Journal of Expermental Biology. 207:187–195.

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