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The efficiency of *Bt* corn expressing Cry1Ab on biological and histopathological changes of *Sesamia cretica* (Lederer) (Lepidoptera: Noctuidae).

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Abstract: The pink stem borer *Sesamia cretica* is considered the most serious corn borer in Egypt and attacks young maize plants shortly after emergence devours the whorl leaves and may kill the growing points, causing dead hearts. Infested corn plants with *S. cretica* (larval stage) collected from the field and transferred to the laboratory. The larvae of *S. cretica* were reared on maize pieces. Then these larvae were fed on *Bt* corn to investigate the efficiency of *Bt* corn on biological aspects of 1st and 2nd larval instars and on histopathological effects on 4th instar larvae of pink corn borer *S. cretica* compared with non *Bt* corn under laboratory conditions. Results recorded that the *Bt* corn is more effective on 1st and 2nd instar larvae due to the larval mortality percent estimated by 100.0%. *Bt* corn showed highly histopathological disturbance in the epithelium of mid gut of 4th instar larvae.

I. Introduction

In Egypt, corn plants are severely attacked by different species of Lepidopteran pests, referred to as corn borers. The corn borers attacking maize in Egypt are: the pink stem borer *Sesamia cretica* Led. (Noctuidae), the European corn borer (ECB) *Ostrinia nubilalis* Hübn (Pyralidae) and the purple-lined corn borer *Chilo agamemnon* Bles. (Crambidae). The pink stem borer *S. cretica* is considered the most serious corn borer in Egypt and attacks young maize plants shortly after emergence devours the whorl leaves and may kill the growing points, causing dead hearts. It is capable of damaging older plants and excavating tunnels into the stem, ears and/ or cobs. This pest lays its eggs during March, so its larvae cause complete death of small maize plants in April and May causing drastic yield losses (**Mostafa, 1981**; **Simeada, 1985**; **El-Metwally, 1987** and **El-naggar 1991**).

At least ten genes encoding different *Bt* toxins have been engineered into different crops plants: Cry 1 Aa, Cry1Ab, Cry 1 Ac, Cry 1 Ba, Cry1Ca, Cry1H, Cry2 Aa, Cry3A, Cry6A and Cry9C **(Schuler et al., 1998)**. *Bt* toxins are specific to a limited number of insect species with no toxicity against humans or other organisms (**Bravo et al., 2011**).

Recently, corn plants that have been genetically transformed to express the Cry1Ab, Cry1Ac, and Cry9C endotoxins from *Bacillus thuringiensis* (referred to as *Bt* corn) have been developed and are being marketed in the United States and Europe.

The widespread and intensive use of different synthetic insecticides for controlling these pests caused increasing environmental problems including insect resistance, excessive persistence of residues, human health hazards and harmful effect on non-target organisms (**Dahi, 2012**). In 2009, genetically modified corn is grown on about 41.08 million hectares worldwide, which is more 25% of global corn area **(James, 2010).** However, there are concerns that *Bt* corn cultivation could result in adverse effects on the environment. One of the potential adverse environmental effects of *Bt* corn is the non-target effect on soil-dwelling organisms **(Icoz and Stotzky, 2008).**

The present study was devoted to compare between *Bt* corn which genetically modified by *Bacillus thuringiensis* (*Bt*) gene and non *Bt* corn on biological aspects of 1st and 2nd instar larvae of *S. cretica* which reared in these corn varieties and on histopathological effects on 4th instar larvae under laboratory conditions.

II. Materials and Methods

Test insect: Infested corn plants with *S. cretica* (larval stage) were taken from the field and transferred to the laboratory. The larvae of *S. cretica* were reared on maize pieces according to **Aly et al., 2011**.

Bioassay: Laboratory experiments were conducted to study the effect of *Bt* corn on 1st and 2nd instars of *S. cretica* larvae compared with non *Bt* corn.

For treatment, three equal pieces of tender parts stems of *Bt* corn plant were introduced to larvae. The experimented larvae were kept starved for about 4 hours, before offering the food to assure rapid ingestion. Larvae were offered *Bt* corn parts for 24 hours. The total number of treated larvae/ treatment was 30 that were divided in 3 replicates of 10 larvae per each. The same numbers of larvae were considered as a control, these larvae were offered tender parts of maize plant stems immersed in distilled water. Each treatment comprised 30 larvae and was replicated 3 times (larvae jar). The same numbers of larval mortality was determined at the end of the larval stage.

Newly formed pupae were collected on the same day of pupation and weighted then placed in the glass tube (2.5x7.5cm) (one pupae for each tube) and plugged tightly with a piece of cotton. After emergence ten of newly emerged moths were transferred on the same day of emergence to a glass mating-cage as mentioned before, each has 2 adult (♂ & ♀). Daily observations were made to record the adult survival, collect and count the number of deposited eggs. The eggs were incubated at the same conditions.

These tests were carried out to define the larval mortality percentage, larval duration., pupation percentage, pupal weight., pupal duration., pupal mortality percentage, adult emergence, sex ratio, male and female longevity, fecundity (No. of egg laying/female) and fertility % (egg hatchability).

Histopathological effects of *Bt* corn on *S. cretica* larvae:

The 4th instar larvae of *S. cretica* were fed for 24 hrs. on pieces of tender parts of corn plant stems *Bt* corn and non *Bt* corn. From each variety, five larvae were selected were fixed in 10% normal saline. After then washed carefully in water consequently, were dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraplast 58-62°C processed for the formation of paraffin blocks. Serial histological sections 5 microns were cut. Stained with Harris hematoxilin, eosin and investigated under microscope.

III. Results and Discussion

Biological aspects of 1st and 2nd instar larvae of *S. cretica* reared on *Bt* and non *Bt* corn under laboratory conditions:

The present study aims to compare between the effect *Bt* corn and non *Bt* corn on biological aspects (larval duration, pupation, larval mortality, pupal weight, pupal duration, pupal mortality and emergence percentage) of 1st and 2nd instar larvae of *S. cretica.*

The results in Table (1) summarized the efficacy of *Bt* corn against the 1st and 2nd larval instars of *S. cretica* .The data indicate that the percentage mortality of larvae differed widely when larvae of *S. cretica* were fed on *Bt* corn plants after 24hrs of feeding. Larval mortality reached 100% on 1st and 2nd larval instars. The death of all larvae of *S. cretica* after feeding them on small parts of *Bt* corn, could be attributed to the presence of a toxic protein produced in plants as a result of expression of the *Bt* gene introduced via genetic engineering of this maize. These results agree with **Saad El-Deen, 2008,** his study reported that corn cultivar SC Ageeb caused 100% mortality in the larvae of *S. cretica*. Larvae death on Bt corn was also reported by many investigators on many corn borers other than *S. cretica* (**Pilcher et al., 1997 and Lozzia et al., 1998)**.

On contrary, larval mortality percentage reached to 10% and 13% for 1st and 2nd instar larvae respectively when *S. cretica* was fed on non *Bt* corn. The pupation percent was 0.0 and 90% for *Bt* corn and non *Bt* corn respectively for 1st instar larvae. The pupation percent was 0.0 and 87% for *Bt* corn and non *Bt* corn respectively for 2nd instar larvae. It is worth noting that the results of this infestation on larval mortality in the laboratory assured the results recorded in the field, where *Bt* corn was resistant to infestation with *S. cretica.*

Mean larval duration (Table 1) it ranged from 20.56 and 18.88 days for 1st instar larvae and 2nd instar larvae respectively when they were fed on non *Bt* corn. Mean of the rest of reproduction attributes of *S. cretica* did not include those on *Bt* corn, since all larvae fed on this cultivar died, so all successive stages of insect after larvae were absent.

The present study is one of the few attempts in Egypt to evaluate the effect of *Bt* corn against the pink corn borer S. cretica under laboratory conditions. Our findings confirm that the transgenic corn containing a Cry 1Ab gene has significantly more efficacy against *S. cretica* than the conventional corn, for all things measured.

Histopathological changes of *Bt* corn for midgut of *S. cretica* 4th instar larvae:

The mid gut epithelium consists of tall columnar cells and is interspersed apically with the goblet cells and basally with regenerative cells. The columnar cells contain large nuclei in the middle of apical region. The columnar cells possesses a fine brush border facing towards lumen and large number of vesicles discharging into extra peritrophic region of the lumen .The goblet cells are flask shaped with oval nuclei. The peritrophic membrane is well evident in the lumen of the mid gut (**Dahi *et al*., 2011**).

The histopathological observations obtained from histological sections of midgut of 4th instar larvae of *S. cretica* which were fed on *Bt* corn. After 24h post-feeding on 1st generation and 2nd generation of *Bt* corn, showing global loosing of the midgut epithelium cells, absence of cell boundaries and distoration in both muscle

layers and basement membrane as shown on (fig. 2-a,2-b) and (fig. 3-a, 3-b). These results agreed with **Bravo *et al.* (1992)** who determined the damage of different insecticidal crystal proteins (ICPs) from *Bacillus thuringiensis* to the midgut of two lepidopteran (*Manduca sexta* and *Plutella xylostella*) and one coleopteran *Leptinotarsa decemlineata* larvae by light microscopic observations. Results showed that histopathological changes included disruption of the brush border, vacuolization of the cytoplasm, hypertrophy of the epithelial cells, and disintegration of the cell. After ingestion by the insect larvae, the ICPs rapidly accumulate in the peritrophic membrane. In the lepidopteran larvae, the lepidopteran-specific toxic ICPs initially accumulate at the apical microvilli of the epithelial cells in the anterior part of the midgut. In the Colorado potato beetle larvae, CryIIIA is primarily retained by the microvilli of the epithelial cells from the posterior part of the midgut.

To kill lepidopteran pests, *Bt* toxins must be ingested by caterpillars, become activated by gut proteases, and then bind to midgut receptors. Two mechanisms have been proposed for the subsequent steps in the toxins' mode of action: (i) pore formation in midgut epithelial cells followed by colloid-osmotic lysis or (ii) activation of a signaling cascade after binding to a primary target in the midgut.(**Zhang et al., 2006, Soberon et al., 2009, Bravo et al., 2011). Rouis et al. (2007)** investigated the mode of action of Cry insecticidal toxins of *Bacillus thuringiensis kurstaki* in *Prays oleae* midgut. Results showed evidencing a midgut columnar cell vacuolization, microvilli damage, and then a pass of epithelium cell content into the larvae midgut. Moreover, Bacillus thuringiensis toxins were shown to bind to the apical microvilli of the midgut epithelial cells.

Also results in harmony with **Pinto et al. (2010)** who evaluated purified Cry1Ba protein in the midgut epithelial cells of *Spodoptera frugiperda* larvae. The histopathological analysis showed a progressive loss of epithelial cell definition after 3h, in both treatments. At 24h post treatment, their midgut changes included vacuolization of the cytoplasm, hypertrophy of the epithelial cells, and vesicle formation in the apical region of both goblet and columnar cells. Also, the brush border membrane was damaged, especially in goblet cells. Also **Bravo et al. (2013**) reported that 3D-Cry toxins are recognized as pore forming toxins that kill larval epithelium midgut cells by causing an osmotic shock leading to cell lysis.

Table (1): Effect of *Bt* corn on larval stage of 1st and 2nd instars larval of *S. cretica*

Biological aspects	1st instar		2nd instar	
	Bt corn	Control	*Bt* corn	Control
Larval duration (days ± S.E)	—	20.56 ± 0.39	—	18.88 ± 0.46
Pupation %	0.0	90 %	0.0	87 %
Larval mortality %	100.0	10 %	100.0	13 %

Table (2): Effect of *Bt* corn on pupal stage of 1st and 2nd instars larval of *S. cretica*

Biological aspects	1st instar		2nd instar	
	Bt corn	Control	*Bt* corn	Control
Pupal weight (gm)	—	0.1682± 0.01	—	0.1668± 0.01
Pupal weight ♂ (gm)	—	0.1490± 0.00	—	0.1489± 0.00
Pupal weight ♀ (gm)	—	0.1907± 0.01	—	0.1800± 0.01
Pupal duration (days ± S.E)	—	9.32± 0.19	—	9.73± 0.2
Pupal duration ♂ (days ± S.E)	—	9.43± 0.29	—	10.00± 0.27
Pupal duration ♀ (days ± S.E)	—	9.18± 0.23	—	9.53± 0.29
Pupal mortality %	—	7 %	—	0.0 %

Table (3): Effect of *Bt* corn on adult stage of 1st and 2nd instars larval of *S. cretica*

Biological aspects		1st instar		2nd instar	
		Bt corn	Control	*Bt* corn	Control
Emergence%		—	93 %	—	100 %
Sex ratio %	♂	—	56 %	—	42 %
	♀	—	44 %	—	58 %
Fecundity (No. eggs/♀)		—	138.0 ± 39.58	—	120.0 ± 43.09
Pre-oviposition		—	5.0 ± 1.73	—	4.0 ± 1.53
Oviposition		—	3.67 ±1.33	—	3.33 ± 0.88
Post-oviposition		—	0.0	—	0.0

Table (4): Effect of *Bt* corn on egg stage of 1st and 2nd instars larval of *S. cretica*

Biological aspects		1st instar		2nd instar	
		***Bt* corn**	**Control**	***Bt* corn**	**Control**
Hatchability %		—	**61 %**	—	**72 %**
Incubation periods (days ± S.E)		—	**5.03 ± 0.02**	—	**5.28 ± 0.06**
Longevity (days±S.E)		—	**9.33 ± 0.61**	—	**9.5 ± 0.56**
Longevity (days ±S.E)	♂	—	**9.0 ±1.0**	—	**10.0 ± 0.58**
	♀	—	**9.67 ± 0.88**	—	**9.0 ± 1.0**

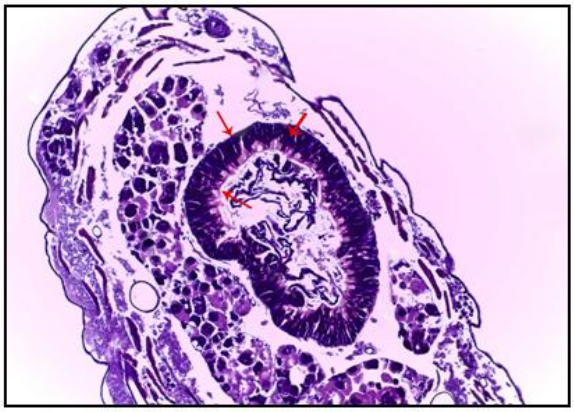

Fig (1-a): Transverse section in the midgut of untreated 4th instar larvae of *S. cretica* [10 X].

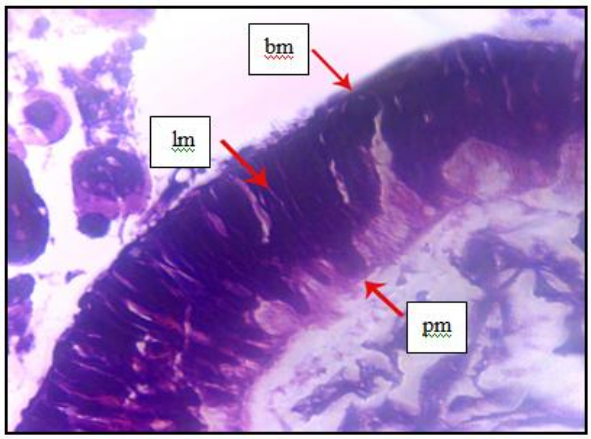

Fig (1-b): Transverse section in the midgut of untreated 4th instar larvae of *S. cretica* [40 X] peritrophic membrane (pm), longitudinal muscle (lm) and basement membrane (bm).

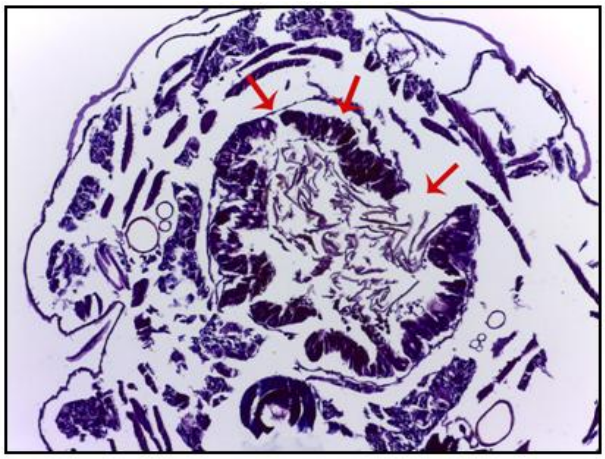

Fig (2-a): Transverse section in the midgut of 4th instar larvae of *S. cretica* after 24 hrs. post feeding on 1st generation of *Bt* corn [10 X].

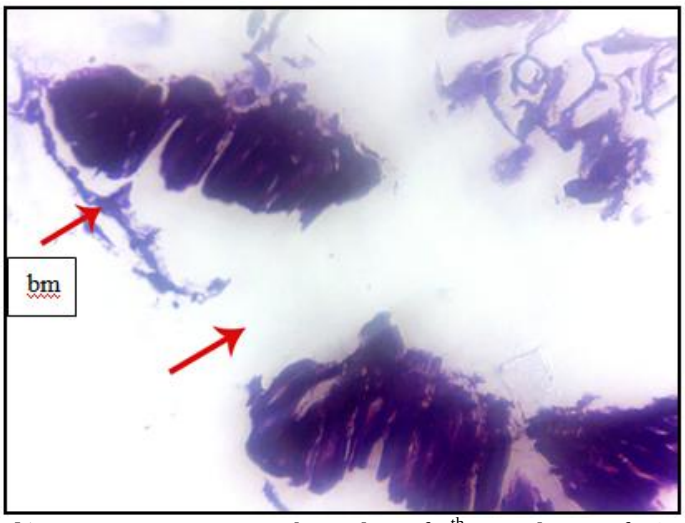

Fig (2- b): Transverse section in the midgut of 4th instar larvae of *S. cretica* after 24 hrs. post feeding on 1st generation of *Bt* corn [40 X].

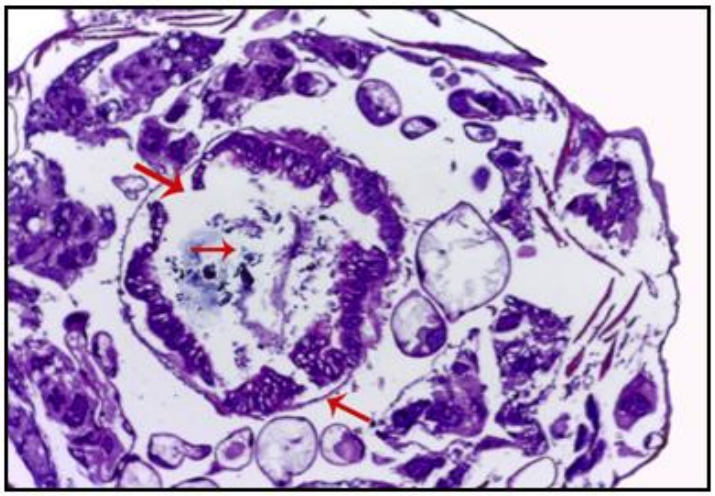

Fig (3, a): Transverse section in the midgut of 4th instar larvae of *S. cretica* after 24 hrs. post feeding on 2nd generation of *Bt* corn [10X].

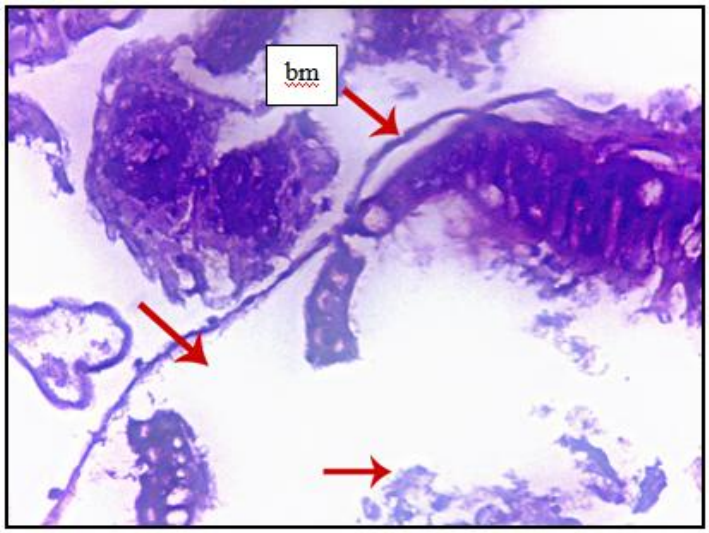

Fig (3, b): Transverse section in the midgut of 4th instar larvae of *S. cretica* after 24 hrs. post feeding on 2nd generation of *Bt* corn [40 X].

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