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Susceptibility of the Egyptian Cotton Leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) to Entomocidal Crystal Proteins Cry1Ac and Cry 2Ab Baseline Responses

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ABSTRACT

The present study maybe is the first attempt in Egypt to evaluate the effect of Entomocidal proteins (Cry toxins) from *Bacillus thuringiensis* (*Bt*) Cry 1Ac and Cry 2Ab against the Egyptian cotton leafworm *Spodoptera littoralis.* The cotton leafworm one of the important Lepidopteran species is the most important pests in cotton fields. It can be controlled using Genetically Modified (GM) crops expressing insecticidal *Bt* proteins like *Bt* cotton and *Bt* corn. The long-term success of this technology demands pest resistance management. Important information for the successful management of resistance is the baseline susceptibility of the different target pests to the different *Bt* proteins. The data on baseline susceptibility should enable risk assessors and managers to assess whether GM cotton produces a *Bt* protein in a high-dose to specific target organisms and resistance has evolved during the commercial cultivation of this GM cotton event. During this study, the toxicity of Cry 1Ac and Cry 2Ab from the bacterium *Bacillus thuringiensis* to larvae of *S. littoralis* was assessed using first instar larvae with diet incorporation bioassays. Larvae reared (4 generations) from 2 populations of *S. littoralis* collected from major cotton-growing regions of Sohag and Assuit Governorates were tested. The LC_{50} for the Assuit strain was 140.11 and 88.54 µg/ml for Cry 1Ac and Cry 2Ab, respectively. While, the LC_{50} for the Sohag strain was 410.75 and 37.62 µg/ml for Cry 1Ac and Cry 2Ab, respectively. On the other hand, the laboratory strain was more sensitive for the same Cry's (Cry 1Ac and Cry 2Ab) the LC_{50} was13.40 and 0.23 µg/ml, respectively. The lethal concentration (LC_{50}) against *S. littoralis* for 2nd and 4th instars larvae when using the *B. thuringiensis* as spores (commercial product) was 1.16 and 2.49 for 2nd and 4th instars, respectively. This study is very important as a preliminary step and baseline ahead of the commercial cultivation and production of Genetically Modified cotton (*Bt* cotton).

INTRODUCTION

The Egyptian cotton, *Gossypium barbadense* L., is considered a major economic crop in Egypt; it represents the first cash crop for national income. Thus, the governmental

policy in Egypt is offering all facilities to encourage farmers to increase the production of cotton in order to meet the increasing requirements for local production and allow surplus for exportation. Several million pounds are paid every year for controlling the cotton pests, the control of *S. littoralis* based mainly on foliage treatments with chemical synthetic insecticides. The widespread and intensive use of different synthetic insecticides for controlling this pest caused increasing environmental problems including insect resistance, excessive persistence of residues, human health hazards and harmful effects on non-target organisms (Dahi, 2012). *Bacillus thuringiensis* (*Bt*) is a Gram-positive, soil-dwelling bacterium. Many *Bt* strains produce insecticidal crystal proteins (Cry proteins) during sporulation, as well as vegetative insecticidal proteins (Vips) during vegetative stages of growth (Estruch *et al.*, 1996; Schnepf *et al*., (1998) and Chakroun *et al*., (2016). Since 1996, some of these insecticidal *Bt* proteins (mainly Cry proteins) have been incorporated into transgenic crops (*Bt* crops) for control of several major pests of lepidoptera and coleopteran. In 2015, more than 84 million hectares of *Bt* cotton, corn, and soybean were planted globally James, 2015. The toxins of *Bacillus thuringiensis* (*Bt*) have shown great potential in the control of harmful insects affecting human health and agriculture, used as the main biological agent for the formulation of bioinsecticides due to its specificity to target different insect orders (David, *et al*. 2019). The basis of *Bt* insecticidal activity comes from the δ-endotoxins formed during sporulation (Shelton *et al.*, 2002). After ingestion in the midgut of pests, the *Bt* protoxin is activated by the gut proteases into an active toxin, which binds to specific receptors like cadherin and amino peptidase-N or alkaline phosphates in the per trophic membrane, forming pores in the midgut epithelial cells (Fortier *et al*. 2007; Abdullah *et al.*, 2009; and Talaei-Hassanloui *et al.*, 2014). At least ten genes encoding different *Bt* toxins have been engineered into different crops plants: Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ba, Cry1Ca, Cry1H, Cry2 Aa, Cry3A, Cry6A and Cry9C (Schuler *et al.* 1998) and most of the commercial transgenic cotton express Cry1Ac (Luttrell *et al.* 1999; Perlak *et al.*, 2001; Dutton *et al.*, 2002 and Baur and Boethel, 2003). Genetically modified plants expressing insecticidal proteins from the bacterium *Bacillus thuringiensis* Berliner (*Bt*) have been developed and used in agricultural systems to protect crops against damage from the targeted insect pests. The commercial use of the *Bt* cotton product Bollgard expressing the insecticidal protein Cry1Ac (δ-endotoxin) was approved by the National Biosafety Technical Commission in Brazil in 2005 Albernaz *et al.*, 2012. Cry2Ab is toxic only to lepidopteran insects (Höfte and Whiteley 1989; Widner and Whiteley 1989; Dankocsik *et al.* 1990). Cry1Ac has provided significant environmental and economic benefits since its introduction in China in 1997 for control of major lepidopteran pests (Huang, *et al.*, 2002; Lu, *et al.*, 2012; Wu and Guo, 2005; Wu, *et al.*, 2008).

MATERIALS AND METHODS

Insect Rearing:

Two laboratory strains of *S. littoralis* were obtained from Plant Protection Research Institute, Cotton Leafworm Department, Dokki, Giza. The first strain was fed on castor oil plants *Ricinus communis* and the rearing technique was according to Dahi, 1997 as follows:

Egg masses of *S. littoralis* were resulting from a maintained insect culture reared in the laboratory for at least four generations on the fresh leaves of castor oil plant as natural food which resemble the natural food in the field (i.e., cotton leaves). For strain establishment, the eggs were maintained at 27 ± 2°C and 65 ± 5 % R.H until hatching. Newly hatched larvae were transferred to clean 5-pound glass-Jars covered with muslin and secured with rubber bands. They were provided with fresh castor-oil leaves which were renewed daily until the larvae show the signs of pupation. A thin layer of fine saw-dust was spread

on the bottom of every glass-Jars to help the successful pupation. Pupae were kept individually in a vial until moth emergence. Ten pairs of newly - emerged moths were confined into oviposition cages. Which consists of conventional mating glass bells (16cm. high and 8cm.diam.) opened at each end. Each mating-glass bell was supplied with a small fresh branch of *Nerium oleander* to serve as an oviposition site and placed on its wide end on a half petri-dish. Tops of the glass bells were covered with muslin and secured with rubber bands. Cages were examined daily to replace *N. oleander* branches with new ones and renew the adult feeding solution (a small piece of absorbent cotton wool previously soaked in 10% sucrose solution). The cages were maintained at the same conditions of temperature and % R.H. Deposited egg- masses were kept in petri-dishes, and then, were available to achieve the different experiments for the toxicity of *Bt* spores (Protecto®) against 2nd and 4th instar larvae of *S. littoralis* laboratory strain. The second strain: the larvae were fed on an artificial diet according to Abdel-Hafez *et al.*, (1982). The rearing technique was according to Dahi, 2005. Newly hatched larvae were fed individually in glass vials (20 x 7.5 cm) filled to one-third with the artificial diet, covered with absorbent cotton and held in the same conditions as mentioned above. The larvae pupate usually on the diet's top or between its parts. At the beginning of pupation, the vials were examined daily for transferring the formed pupae individually to clean vials and incubated until moth emergence. Ten pairs of newly emerged cotton leafworm moths were confined in glass oviposition, which consists of a conventional mating glass bell (16 cm. high and 8cm. diam). opened at each end. Each mating - glass bell was supplied with a small fresh branch of *Nerima oleander* to serve as an oviposition site and placed on its wide end on a half petri-dish. Tops of the glass bells were covered with muslin and secured with rubber bands. Cages were examined daily to replace *N. oleander* branches with new ones and renew the adult feeding solution (a small piece of absorbent cotton wool previously soaked in 10% sucrose solution). The cages were maintained at the same conditions of temperature and % R.H. deposited egg- masses were kept in petri dishes, and then was available to achieve the different experiments for the toxicity of Cry 1Ac and Cry 2Ab against newly hatched larvae of *S. littoralis* Assuit and Sohag field strains and laboratory strain.

Field strains of *S. littoralis* egg masses were collected from Assiut and Sohag Governorates. Then transferred to the laboratory and rearing at least four generations at 27 ± 2 °C and 65 ± 5 % R.H to become sensitive strains on the semi-artificial diet by the method described as mentioned before.

Tested Compounds:

1- **Bio-insecticide** (Protecto) used as a commercial product of *Bacillus thuringiensis* subsp. *Kurstaki* contains 32x10^3 I. U/mg). Active ingredient 9.4% was obtained from Plant Protection Research Institute, Agricultural Research Centre, Egypt.

2- **Cry1Ac and Cry2Ab toxins** were freeze-dried powders obtained from Monsanto Company, USA.

Cry1Ac and Cry2Ab protoxin is a crystal protein produced by the gram-positive bacterium, *Bacillus thuringiensis (Bt)* during sporulation. Transgenic *Bt* cotton expressed Two *Bt* gene, which codes for Cry1Ac and Cry2Ab. Transgenic crops expressing the insecticidal proteins Cry1Ac and Cry2Ab from *Bacillus thuringiensis (Bt)* are used worldwide to suppress damage by lepidopteran pests, often used in combination between Cry1Ac and Cry2Ab toxin to delay resistance evolution in *Bt* cotton.

Bioassaying Test:

To evaluate the LC_{50} of *B. thuringiensis* Protecto against 2nd and 4th instars larvae of the cotton leafworm, *S. littoralis* by leaf dipping technique. Serial concentrations of *B. thuringiensis* were prepared based on ppm water from (20, 15, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078) for each instar. Five replicates (each of 10 larvae/concentrations) were

used. All the castor leaves were dipped for 30 seconds in each concentration, then left to dry under laboratory conditions. Larvae were allowed to feed on treated castor bean leaves (*Ricinus communis* L.,) for 48 hours then allowed to feed on untreated leaves. Data were recorded after 2, 3, 5, 7 days and the percentage of mortality was estimated.

Two field strains collected from Assiut and Sohag of *S. littoralis* in 2020 were evaluated for susceptibility to Cry1Ac and Cry2Ab. While concentrations (1500, 1000, 500, 100, 4, 0.8 µg/ml) and (1250, 1000, 500, 100, 20, 0.8 µg/ml) were used for Cry1Ac and Cry2Ab, respectively, for Assiut Strain. Concentrations (1500, 750, 500, 100, 20, 4 µg/ml) and (1000, 500, 100, 20, 4, 0.8 µg/ml) were used for Cry1Ac and Cry2Ab, for Sohag Strain, respectively. Additionally, the susceptibility of a laboratory strain of *S. littoralis* was evaluated at the same time was being tested. Serial concentrations of Cry toxins of Cry1Ac and Cry2Ab were prepared by diluting with an artificial diet. Cry1Ac were (750, 500, 100, 20, 4, 0.8, 0.16, 0.32 µg/ml) and concentrations (500, 100, 20, 4, 0.8, 0.16, 0.32, 0.0064, 0.00128 µg/ml) were used for Cry2Ab. Three replicates were used for each of the 20 larvae/concentrations. Control larvae were feed on an artificial diet without toxins. After mixing the toxin with diet (diet-incorporation), neonate offspring were transferred individually with brushes into 1 oz plastic cups. These cups had tight-fitting lids and contained about 5 gm of the diet each. One neonate larva was placed into each cup and the tops were affixed.

A 7-day diet-incorporation bioassay (Wei *et al.*, 2017) was used. After 7-day neonates that developed to ≥ the 4th instar stage (Watson and Johnson 1974) were scored as alive and the percentage of mortality was estimated and corrected using Abbott's correction (Abbott 1925). Estimates of LC_{50}, and corresponding 99% confidence limits, were calculated using probit analysis Finney (1971).

Biological Aspect:

The larval instars which treated with LC_{50} of the *B. thuringiensis*, Cry1Ac and Cry2Ab were observed for the following biological aspect: larval duration, pupation rate, larval mortality percentage, the pupal duration, pupal weight, percentage, sex ratio, malformation, intermediate shape, emergence, and pupal mortality, female and male longevity, adult longevity, pre-oviposition, oviposition, post-oviposition period, fertility % and Incubation period.

RESULTS AND DISCUSSION

Toxicity of *B. thuringiensis* as spores against 2nd and 4th instars larvae of *S. littoralis* under laboratory conditions.

The toxic action as the initial toxic effect of the investigated compound to the 2nd and 4th instars larvae of *S. littoralis* was evaluated under laboratory conditions. The main criteria of the toxicity regression line, LC_{50} and slope value were used as a parameter to determine the level of toxicity for *B. thuringiensis* when using it as spores (commercial product).

Data summarized in Table (1), showed that the toxic effect of *B. thuringiensis* spores represented as LC_{50}'s against *S. littoralis* for 2nd and 4th instars. LC_{50} was 1.1631 and 2.498 for 2nd and 4th instars, respectively, (Figs. 1 & 2). Harapaz and Wysoki (1984) found that 1% concentration of *B. thuringiensis* wettable powder (containing 16.000 lU/mg) applied at a rate of 48.000 IU/cm2, killed 95 % of 4th instar larvae of carob moth, *Ectomyelcis ceratoniae* (Zeller) (Lepidoptera: Pyralidae), after 66 h. Meanwhile, 100% mortality was recorded after 85 h of exposure in a laboratory test. The mortality caused by 0.5 % concentration (24.000 lU/cm2) was significantly lower and presumably inadequate for practical application against this pest. Yamamoto *et al.*, (1990) evaluated the effect of *B. thuringiensis*, cyfluthrin and fenvalerate on the larvae of *Alabama argillacea*. Fenvalerate gave 60 % control and *B.*

thuringiensis was the least effective controlling less than 50 % up to 6 days after application. A significant reduction in the pupal population 6 days after application of *B. thuringiensis* indicated some secondary effect on the larvae before pupation. Also, Mohamed *et al.,* 2011, studied the toxic effects of entomopathogenic bacterium, *Bacillus thuringiensis* subsp. Kurstaki on the larval instars of the *Sesamia cretica* (Lederer) and the same trend for toxic effect was observed.

Table 1: Toxicity of *B. thuringiensis* as spores (commercial product) against the 2nd and 4th instars larvae of *S. littoralis*

Biocide	Larval instar	LC_{50} (ppm)	Slope
B. thuringiensis (Protecto®)	2nd	1.1631	0.0835
	4th	2.498	0.1256

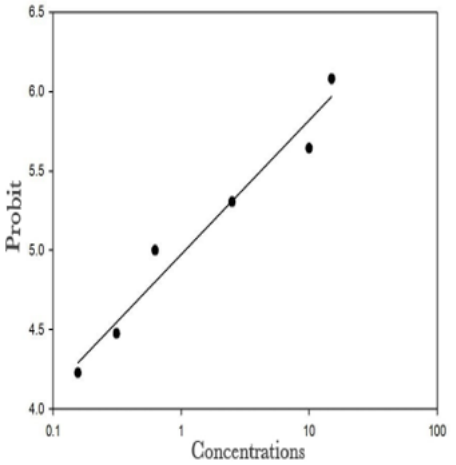

Fig.1: Toxicity line of *Bt* spores against *S. littoralis* 2nd instar larvae

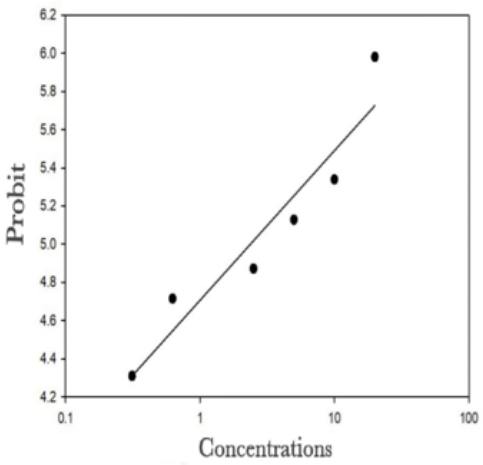

Fig.2; Toxicity line of *Bt* spores against *S. littoralis* 4th instar larvae

Toxicity of Cry1Ac and Cry2Ab against Newly Hatched Larvae of *S. littoralis* Field and Laboratory Strains.

Data presented in Table (2), show that the efficacies of tested *Bt* toxins represented as LC_{50}'s against *S. littoralis* after 7 days. For Cry1Ac: Sohag strain was less susceptible than Assiut strain. The LC_{50} of Sohag strain was 410.75 µg/ml, while, the LC_{50} of Assiut strain was 140.11 µg/ml. (Figs. 5 &7).For Cry2Ab: Sohag strain was more susceptible than Assiut strain. The LC_{50} of Assiut strain was 37.620 µg/ml while the LC_{50} of Sohag strain was 88.539 µg/ml. (Figs. 6 & 8).For laboratory strain: Cry2Ab was higher effectiveness than Cry1Ac. (Figs. 3 &4).Yiyun *et al.,* (2017) studied the same point on *Helicoverpa armigera* in China, the study reported the control of this pest has been dependent on transgenic cotton producing a single *Bacillus thuringiensis* (*Bt*) protein Cry1Ac since 1997. A small, but significant, increase in *H. armigera* resistance to Cry1Ac was detected in field populations from Northern China. Since Vip3Aa has a different structure and mode of action than Cry proteins, *Bt* cotton pyramids containing Vip3Aa are considered as ideal successors of Cry1Ac cotton in China. In this study, the baseline susceptibility of *H. armigera* to Vip3Aa was evaluated in geographic field populations collected in 2014 from major cotton-producing areas of China. The LC_{50} values of 12 field populations ranged from 0.053 to 1.311 µg/cm^2, representing a 25-fold range of natural variation among populations. It is also demonstrated that four laboratory strains of *H. armigera* with high levels of resistance to Cry1Ac or Cry2Ab have no cross-resistance to Vip3Aa protein. The baseline susceptibility

data established here will serve as a comparative reference for the detection of field-evolved resistance to Vip3Aa in *H. armigera* after future deployment of *Bt* cotton pyramids in China. Unnithan *et.al.*, 2004, studied the *Susceptibility* of pink bollworm (PBW), *Pectinophora gossypiella*, to the *Bt* toxin Cry2Ab was evaluated on collections made in the Southwestern U.S. in 2001 and 2002 and contrasted with laboratory strains. PBW were collected from cotton fields, cultured in the laboratory, and tested using diet-incorporation bioassays. A total of 6 collections in 2001 and 14 collections in 2002 were successfully reared and bioassayed. Significant differences between strains in susceptibility to Cry2Ab were found each year, though the range of susceptibility was substantially greater in 2001 than in 2002. LC_{50} estimates of strains ranged from 0.220 to 4.56 µg Cry2Ab/ml and 0.0840 to 0.723 µg Cry2Ab/ml in 2001 and 2002, respectively. Only one field strain, a collection from Tornillo, Texas, was substantially less susceptible to Cry2Ab than was the laboratory strain, AZP-R, which was highly resistant to Cry1Ac. Based on these baseline responses, concentrations of 1.0 and 10 µg Cry2Ab/ml diet have been identified for routine monitoring of pink bollworm. Selection of 2001 and 2002 strains with Cry2Ab is underway in the laboratory but has not yielded intense resistance to Cry2Ab. Thus, at this time we have no indication of significant resistance of Southwestern pink bollworm to the second *Bt* toxin being deployed in transgenic insecticidal cotton.

Table 2: Toxicity of Cry1Ac and Cry2Ab against newly hatched larvae of *S. littoralis* field and laboratory strains.

Strains	Cry Toxin	LC_{50} (µg/ml)	Slope
Assiut strain	Cry1Ac	140.11	0.1592
	Cry2Ab	88.54	0.1757
Sohag strain	Cry1Ac	410.75	0.2910
	Cry2Ab	37.62	0.1148
Laboratory strain	Cry1Ac	13.40	0.1071
	Cry2Ab	0.23	0.0255

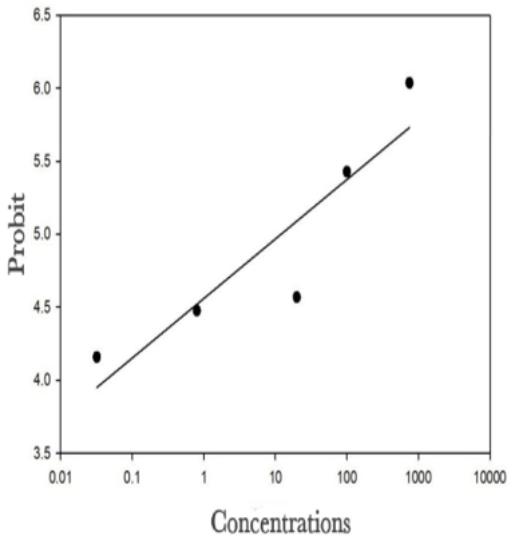

Fig. 3: Toxicity line of Cry1Ac against *S. littoralis* laboratory strain

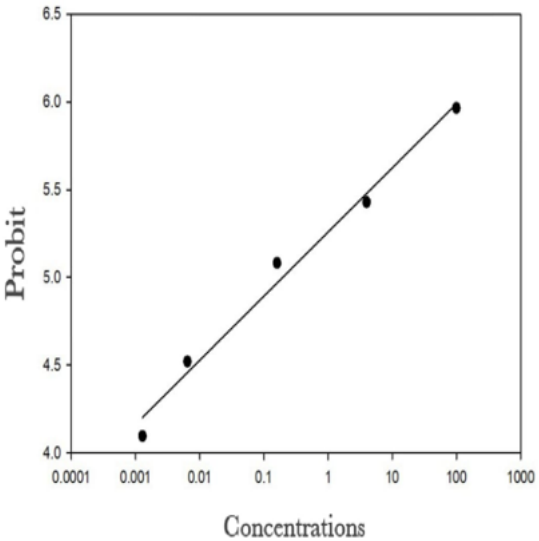

Fig. 4: Toxicity line of Cry2Ab against *S. littoralis* laboratory strain

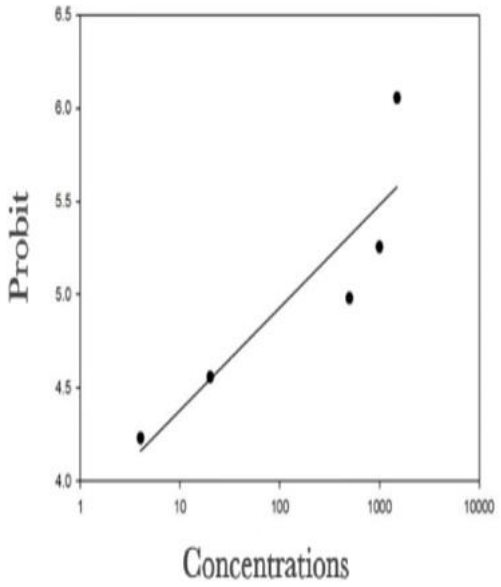

Fig. 5: Toxicity line of Cry1Ac against *S.littoralis* Assiut strain

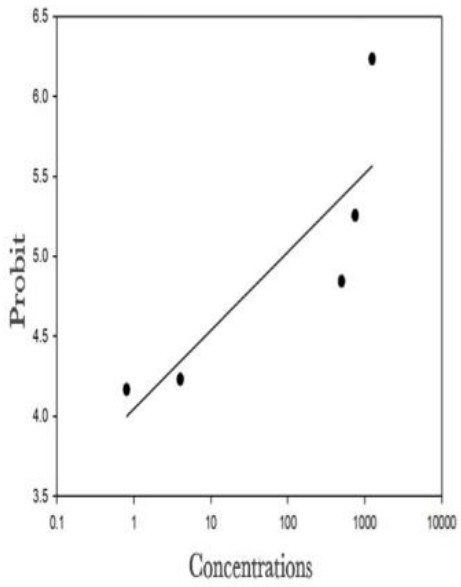

Fig. 6: Toxicity line of Cry2Ab against *S.littoralis* Assiut strain

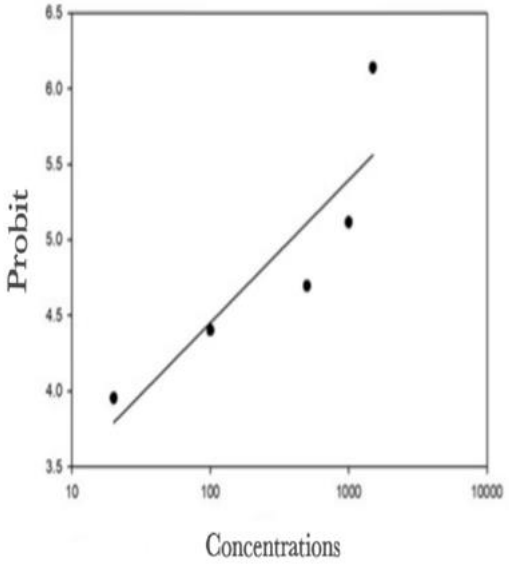

Fig. 7: Toxicity line of Cry1Ac against *S.littoralis* Sohag strain

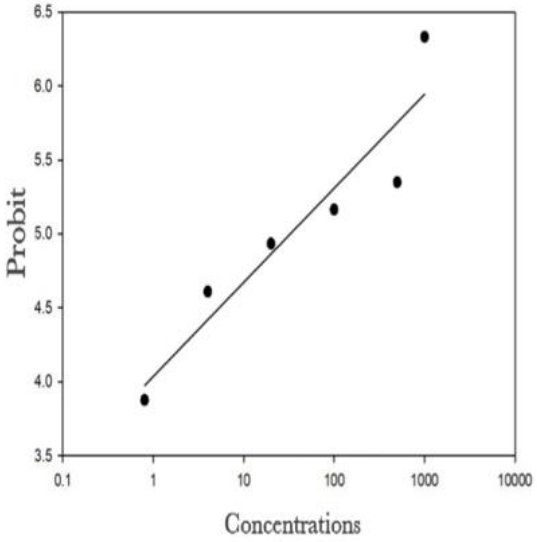

Fig. 8: Toxicity line of Cry2Ab against *S.littoralis* Sohag strain

Effects of *B. thuringiensis* (*Bt*) As Spores on Biological Aspects for *S. littoralis:*

Data in Table (3) show that the biological aspects for 2nd and 4th instars larvae of *S. littoralis* after treated with *Bacillus thuringiensis* (*Bt*) spores. For the 2nd instar larvae: the larval duration was 14.85 days compared with control (15.56 days). The pupation % and larval mortality % were 48.0 and 52.0 compare with control 98.0 and 2.0 %, respectively. The pupal duration**,** pupal weight and sex ratio were 10.3, 0.2262 and 50:50 compare with control 8.78 days, 0.2276 gm and 46.9: 53.1 ♀: ♂, respectively. While, the percentages of malformation**,** intermediate shape, emergence and pupal mortality were 24.0, 4.4, 93.75 and 6.25 % compare with control 2.04, 0.0, 100.0 and 0.0 %, respectively**.** On the other hand, for the adult stage, the female and male longevities were 9.0 and 7.0 compare with the control 9.33 and 8.33 days, respectively. The female pre-oviposition period, oviposition period and post oviposition period were 1.66, 4.66 and 2.66 compared with control 2.0, 4.66 and 2.66 days, respectively. The fecundity (No. egg / ♀) and fertility % for *S. littoralis* when the

larvae treated in 2nd instar were 844.66 and 92.0 compared with control 1961.33 egg / ♀ and 100.0, respectively. The adult longevity was 8.0 and 8.33 days for treatment and control, respectively. The incubation period for egg stage at the next generation after treatment *S. littoralis* 2nd instar larvae with *Bt* spores was 3.43 compared with control 3.38 days. For the 4th instar larvae: the larval duration was 9.35 days compared with control (10.57 days). The pupation % and larval mortality % were 58.0and 42.0 compare with control 96.0 and 4.0 %, respectively. The pupal duration**,** pupal weight and sex ratio were 8.71, 0.2249 and 53.57:49.0: 46.43 compare with control 8.12 days, 02301 gm and 49.0:51.0 ♀: ♂, respectively. While, the percentages of malformation**,** intermediate shape, emergence and pupal mortality were 20.68, 3.44, 100.0 and 0.0 % compare with control 0.0, 0.0, 100.0 and 0.0 %, respectively. On the other hand, for the adult stage, the female and male longevities was 8.33 and 7.33 compared with control 9.32 and 8.66 days, respectively. The female pre-oviposition period, oviposition period and post oviposition period were 1.0, 4.00 and 3.33 compared with control 1.9, 4.76 and 2.66 days, respectively. The fecundity (No. egg / ♀) and fertility % for *S. littoralis* when the larvae treated in 4th instar were 1556.0 and 95.0 compared with control 1709.0 egg / ♀ and 100.0, respectively. The adult longevity was 7.82 and 8.32 days for treatment and control, respectively. The incubation period for egg stage at the next generation after treatment *S. littoralis* 4th instar larvae with *Bt* spores was 3.38 compared with control 3.07 days. Mona *et al.,* (2011) clear that 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 LC50 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 was 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 was 60.3, 60.4 and 61.3 % for Agerin, Dipel 2X and Dipel DF, respectively. On the other hand, Mohamed *et al.,* (2011), reported that The LC_{50} value after 48 hours from treatment, of *B. thuringiensis* for 1st instar and 2nd larvae were 0.00526 and 0.7 gm, respectively. After treatment of 1st and 2nd instars larvae of *S. cretica* with LC_{50} concentrates of *B. thuringiensis*, the average larval duration of *S. cretica* were (36.53 & 24.69) and (33.78& 20.31) days for treated and untreated, respectively. In addition, the pupation percentage was (47 & 92 %) and (18 & 84 %) for treated and untreated, respectively. The larval mortality percentage was (53 & 8 %) and (82 & 16 %) for treated and untreated, respectively. The pupal weight for (treated & untreated), S. cretica were (0.1887 & 0.184 gm) and (0.1842 & 0.1601 gm) when it treated as 1st and 2nd instars larvae, respectively. Furthermore, the pupal duration were (12.14 & 10) and (10.44 & 9.83 days) for (treated & untreated), when *S. cretica* was treated as 1st and 2nd instar larvae, respectively. The total adult emergence for 1st instar and 2nd instar larvae were (94 & 100%) and (100 & 100%) for treated and untreated, respectively.

Effects of Cry1Ac and Cry 2Ab Toxins on biological aspects for *S. littoralis:*

Data in Table (4) show that the biological aspects for newly hatched larvae of *S. littoralis* after feeding on protein toxins Cry1Ac and Cry 2Ab (diet-incorporation). The *S. littoralis* larval duration was 23.62, 25.42 and 17.73 days for Cry1Ac, Cry 2Ab and control, respectively. The pupation % was 41.0, 43.0 and 99.0 % for Cry1Ac, Cry 2Ab and control, respectively, while the larval mortality were 59.0, 57.0 and 1.0 % for Cry1Ac, Cry 2Ab and control, respectively. For pupal stage, the pupal duration **(**days**)** and pupal weight (gm) were 10.29 and 0.2256 for Cry1Ac; 9.39 and 0.2256 for Cry 2Ab and 9.65 days and 0.2276 gm for control, respectively. The pupal mortality was 7.32, 9.50 and 1.0 % for Cry1Ac, Cry 2Ab and control, respectively. For the adult stage: the sex ratio (♀: ♂) were 48.48: 51.52, 52.56:47.22 and 41.41:58:59 for Cry1Ac, Cry 2Ab and control, respectively, respectively.

While, the percentages of malformation**,** intermediate shape, emergence were 26.32, 13.16 and 92.68 % for Cry1; 20.51, 7.69 and 90.69 % for Cry 2Ab and 4.04, 1.01 and 99.0 % for control, respectively. On the other hand, for the adult stage, the female, male and adult longevities were 8.33, 7.0 and 7.67 days for Cry1Ac, 8, 98, 8.67 and8.83 days for Cry 2Ab and 10.43, 8.33 and 9.38 days for control, respectively. The female pre-oviposition period, oviposition period and post oviposition period were 1.33, 5.00 and 2.00 for Cry1Ac, 1.66, 4.66 and 2.66 days for Cry 2Ab and 1.77, 6.00 and 2.66 days for control, respectively. The fecundity (No. egg / ♀) and fertility % for *S. littoralis* when the new hatched larvae feed on diet-incorporation (7- days) with *Bt* two toxins were 1895.7 and 89.0 (for Cry 1Ac), 1716.7 and 85.0 (for Cry 2Ab) and 2276.0 egg / ♀ (for control), respectively. The incubation period for the egg stage of *S. littoralis* in the next generation when diet-incorporation (7- days) with *Bt* two toxins was 3.47, 3.65 and 3.30 days for Cry1Ac, Cry 2Ab and control, respectively. On the other hand, in South Africa Britz *et al.,* (2020), studied the susceptibility of *Spodoptera littoralis* to *Bt* cotton, expressing Cry1Ac and Cry2Ab Toxins, and reported that the bioassays conducted with *S. littoralis* in Egypt (Dahi, 2012) showed that the effects of these Cry proteins on growth, development and metamorphosis of *S. littoralis* were similar to those observed in this study. This author also reported that the pest was highly susceptible to *Bt* cotton and that there was no larval survival after five days of feeding on Giza 80 cotton that expresses both Cry1Ac and Cry2Ab toxins.

Table 3: Biological aspect of *Spodoptera littoralis* 2nd and 4th instars larvae after feeding on *Bacillus thuringiensis* (*Bt*) spores.

Biological aspect	2nd instar larvae		4th instar larvae	
	Treatment	Control	Treatment	Control
Larval duration (days)	14.85 ± 0.11	15.56 ± 0.15	9.35 ± 0.21	10.57 ± 0.15
Pupation %	48.0	98.0	58.0	96.0
Larval mortality %	52.0	2.0	42.0	4.0
pupal duration (days)	10.3 ± 0.28	8.78 ± 0.15	8.71 ± 0.26	8.12 ± 0.27
Pupal weight (gm)	0.2262 ± 0.007	0.2276 ± 0.023	0.2249±0.008	0.2301 ±0.023
Sex Ratio ♀: ♂	50:50	46.9:53.1	53.57: 46.43	49.0: 51.0
Malformation %	24.0	2.04	20.68	0.0
Intermediate shape %	4.4	0.0	3.44	0.0
Emergence %	93.75	100.0	100.0	100.0
Pupal mortality %	6.25	0.0	0.0	0.0
Female longevity (days)	9.0 ± 0.47	9.33 ± 0. 58.027	8.33 ± 0.54	9.32 ± 0.27
Male longevity (days)	7.0 ± 0.47	8.33 ± 0.27	7.33 ± 0.27	8.66 ± 0.27
Pre-oviposition period (days)	1.66 ± 0.27	2.0 ± 0.0	1.0 ± 0.0	1.9 ± 0.0
oviposition period	4.66 ± 0.27	4.66 ± 0.27	4.0 ± 0.0	4.76 ± 0.27
Post-oviposition period (days)	2.66 ± 0.27	2.66 ± 0.27	3.33 ± 0.54	2.66 ± 0.27
Fecundity (No. egg / ♀)	844.66	1961.33	1556.00	1709.33
Adult longevity (days)	8.0 ± 0.47	8.83 ± 0.136	7.82 ± 0.62	8.32 ± 0.136
Fertility %	92.0	100.0	95.0	100.0
Incubation period (days)	3.43 ± 0.06	3.38 ± 0.01	3.38 ± 0.14	3.07 ± 0.01

Table 4: Biological aspect of *Spodoptera littoralis* new hatched larvae after feeding on Entomocidal toxic protein Cry1Ac and Cry2Ab

Biological aspect	Cry1Ac	Cry2Ab	Control
Larval duration (days)	23.62 ± 0.35	25.42 ± 0.52	17.73 ± 0.09
Pupation %	41.0	43.0	99.0
Larval mortality %	59.0	57.0	1.0
pupal duration (days)	10.29 ± 0.015	9.36 ± 0.028	9.65 ± 0.087
Pupal weight (gm)	0.2256 ± 0.008	0.2256 ± 0.003	0.2676 ± 0.006
Sex Ratio %♀: ♂	48.48: 51.52	52.56: 47.22	41.41: 58.59
Malformation %	26.32	20.51	4.04
Intermediate shape %	13.16	7.69	1.01
Emergence %	92.68	90.69	99.0
Pupal mortality %	7.32	9.30	1.0
Female longevity (days)	8.33 ± 0.27	8.98 ± 0.00	10.43 ± 0.27
Male longevity (days)	7.0 ± 0.47	8.67 ± 0.27	8.33 ± 0.27
Pre-oviposition period(days)	1.33 ± 0.27	1.66 ± 0.27	1.77 ± 0.27
Oviposition period(days)	5.0 ± 0.47	4.66 ± 0.27	6.0 ± 0.54
Post-oviposition period	2.0 ± 0.0	2.66 ± 0.27	2.66 ± 0.27
Fecundity (No. egg / ♀)	1895. 7	1716. 7	3276.0
Adult longevity (days)	7.67 ± 0.14	8.83 ± 0.14	9.38 ± 0.27
Fertility %	89.0	85.0	100.0
Incubation period (days)	3.47 ± 0.14	3.65 ± 0.11	3.3 ± 0.04

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ARABIC SUMMARY

قابلية دودة ورق القطن المصرية للمبيدات البروتينية الحشرية Cry1Acو Cry2Ab كأستجابات اساسية

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تعتبرهذه الدراسة ربما هي المحاولة الاولي في مصر لتقييم تأثير البروتينات السامة للحشرات *Cry* 1Ac and Cry 2Ab والناتجة من بكتريا الباسيلس ثيورنجنسز ضد يرقات دودة ورق القطن.وتعتبر دودة ورق القطن واحدة من اهم أنواع رتبة حرشفية الاجنحة الهامة وهي من أهم الآفات التي تصيب محصول القطن. ويمكن السيطرة عليها باستخدام المحاصيل المعدلة وراثيا والتي تفرز البروتينات السامة للحشرات مثل القطن المعدل وراثا بجينات بكتريا الباسيلس ثيورنجنسز. ويتطلب نجاح هذه التكنولوجيا على المدى الطويل إدارة مقاومة الآفات. والمعلومات الهامة للإدارة الناجحة للمقاومة هي قابلية خط الأساس baseline للآفات المستهدفة المختلفة لبروتينات بكتريا الـ Bt المختلفة. وينبغي أن تمكن البيانات المتعلقة بالقابلية الأساسية مقيمو المخاطر ومديريها من تقييم ما إذا كان القطن المعدل وراثيا ينتج بروتينات سامة بجرعة عالية لكائنات مستهدفة محددة وما ان كانت قد تطورت المقاومة أثناء الزراعة بشكل تجاري للقطن المعدل وراثيا من عدمة. وخلال هذه الدراسة تم تقييم سمية Cry 1Ac و Cry 2Ab والناتجة من بكتيريا *Bacillus thuringiensis* ضد يرقات دودة ورق القطن باستخدام يرقات الفقس الحديث باستخدام طريقة diet incorporation. تم الاختبار على اليرقات التي تمت تربيتها (4 أجيال معمليا) والتي تم جمعها من مناطق زراعة القطن الرئيسية في محافظتي سوهاج وأسيوط. وكانت قيمة الجرعة السامة النصفية LC_{50} لسلالة اسيوط 140.11 و 88.54 µg/mlلكل من Cry 1Ac و Cry 2Ab ، على التوالي. بينما، كانت الـ LC_{50} لسلالة سوهاج 410.75 و 37.62 µg/ml لكل من Cry 1Acو Cry 2Ab ، على التوالي. من ناحية أخرى، كانت السلالة المعملية أكثر حساسية لنفس البروتينات السامة Cry 1Ac Cry 2Ab حيث بلغت الـ LC_{50} 13.40 و 0.23 ميكروجرام لكل ملليتر، على التوالي. كانت ألـ LC_{50} ليرقات العمر الثاني والرابع لدودة ورق القطن عند استخدام بكتريا الباسيلس كجراثيم (منتج تجاري) 1.16 و 2.49 جزء في المليون على التوالي.وتعبر هذه الدراسة غاية في الاهمية كخطوة اولية وعتبة الاساس baseline التي تسبق زراعة وانتاج القطن المعدل وراثيا بصورة تجارية.