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PURIFICATION, ADSORPTION AND INSECTICIDAL EFFECT OF δ-ENDOTOXIN PRODUCED FROM *BACILLUS THURINGIENSIS* VAR. *KURSTAKI*

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Toxin produced by *Btk* was purified by several steps after production optimization. Purified *Btk* gives a single band 116 kDa in protein sample on SDS-PAGE polyacrylamide gel electrophoresis. The amino acids composition was detected in which proline represented the highest concentration (1299.31567µg/ml), followed by glutamic acid (358.75µg/ml). While NH^{+4}represents 211.82 µg/ml. The adsorption of the toxin of *B. thuringiensis* var. *kurstaki* on kaolinite, montmorillonite, goethite, silicon dioxide (SiO_2), and clay and its desorption from the surface of these minerals were carried out. The adsorbed amount by kaolinite was highest (6.69 µg/ml), followed by montmorillonite (6.02 µg/ml), geothite (5.76 µg/ml), clay (5.60 µg/ml) and silicon dioxide was the lowest (5.08 µg/ml). The purified toxin has been found to be potent against Egyptain cotton leaf worm (*Spodoptera littoralis*) larvae at 4th instar, and deformations of mid-gut tissues of the dead larvae directly were examined by light microscope by comparsion with control. Toxin produced by *Btk* showed an antifungal activity against several fungi as *Aspergillus flavus, Trichoderma viride, Fusarium oxysporum, Alternaria alternata, Penicillum sp., Candida albicans and Aspergillus fumigatus Fresinus* and *Aspergillus candidus Link.*

INTRODUCTION

Bacillus thuringiensis (*Bt*) is a Gram-positive, aerobic, spore-forming, rod-shaped bacterium that produces a parasporal, proteinaceous, crystalline inclusion during sporulation, containing more than one type of insecticidal crystal protein (ICP). ICP is solubilized and hydrolyzed in the midgut of larvae of susceptible insects when ingested, releasing polypeptide toxins that eventually cause death of the larvae (Höfte and Whiteley, 1989& Schnepf *et al.*, 1998).

Bacillus thuringiensis (*Bt*) strain *kurstaki* is active against the Egyptian cotton leaf worm *Spodoptera littoralis*. Toward this target, the delta-endotoxin crystal protoxin genes Cry1C (that encodes an insecticidal protoxin in highly specific to *S. littoralis*) and Cry1Ag (1Ac like) were used (Ibrahim *et al.*, 2008).

Adsorption on soil, and the reversibility of this adsorption is an important aspect of the environmental behaviour of these toxins. The

orientation of the molecule and conformational changes on surfaces may modify the toxicity and confer some protection against microbial degradation. Adsorption will have important consequences for both the risk of exposition of non target species and the acquisition of resistance by target species (Helassa *et al.*, 2009). Adsorption of the toxins from *Btk* or *Btt* on clean clays was affected by the type of cation to which the clays were homoionic. In the clay-size fractions, organic matter increased adsorption whereas Ferrous oxides decreased it, and the mineralogy had a prominent role (Muchaonyerwa *et al.*, 2005).

Yong *et al.* (2008) reported that the adsorption, desorption and anti-ultraviolet light characteristics of the protoxin from *Bacillus thuringiensis* strain WG-001 on montmorillonite, kaolinite, zinc oxide and rectorite were studied. The protoxin was easily adsorbed onto minerals and the adsorption reached equilibrium within 0.5-1.0h (except for rectorite) . The maximum amounts of protoxin adsorbed were in the order: montmorillonite > rectorite > zinc oxide > kaolinite. In the range of pH from 9 to 11 (carbonate buffer), the protoxin adsorbed decreased with increasing pH. The adsorption was not significantly affected by the temperature between 5 and 45°C. Both free and adsorbed protoxin were toxic to larvae of *Heliothis armigera.*

Martins *et al.* (2010) isolated Cry toxins from *Bacillus thuringiensis* (*Bt*) used for insect control, they interact with specific receptors located on the host cell surface and are activated by host protease following receptor binding resulting in midgut epithelial cells lysis. Also, they had cloned, sequenced and expressed a Cry1Ba toxin gene from the *B. thuringiensis* 5601 strain which was previously shown to be toxic to *Anthonomus grandis*, a cotton pest. The Cry1Ba6 protein expressed in an acrystaliferous *B. thuringiensis* strain was toxic to *A. grandis* in bioassays. The binding of Cry1Ba6 toxin to proteins located in the midgut brush border membrane of *A. grandis* was analyzed and found that Cry1Ba6 binds to two proteins (62 and 65 kDa) that showed alkaline phosphatase (ALP) activity. This result is the first report that shows the localization of Cry toxin receptors in the midgut cells of *A. grandis.*

Cry1Ab and Cry1Ac strains and proteins synthesized by *Bacillus thuringiensis thuringiensis* and *B. thuringiensis kurstaki* were assessed in the following phytopathogens: *Rhizoctonia solani*, *Pyricularia grisea*, *Fusarium oxysporum* and *F. solani*, which had their micelial growth decreased after incubation in the presence of the bacterial strains.

The aim of this work was to purify *Btk* toxin and detect the molecular mass and amino acid content, then, test the pure toxin adsorbance on different soil minerals in the lab. The effect of purified *Btk* toxin on the 4th instar larvae of cotton leaf-worm *Spodoptera littoralis* stage was studied to detect the abnormalities of mid-gut tissues of larvae. It is also aimed to evaluate the *Btk* toxin as antifungal substances against some fungi.

MATERIALS & METHODS

Bacterial strain:

Bacillus thuringiensis var. *kurstaki* strain was kindly supplied from the Central Scientific Services Laboratory of the National Research Center, Giza, Egypt. This strain has the capability to produce delta-endotoxins (bi-pyramidal structure shape).

Purification of toxin produced by *Bacillus thuringiensis* var. *kurstaki*:

Suspension of *B. thuringiensis* var. *kurstaki* strain was prepared, and powder was obtained by spray drying. The spray-dried powder was washed three times with double distilled water (ddH_2O). Crystals and spores were removed from the aqueous suspension of the powder by centrifugation (16,000 r.p.m for 15 min), and the pellet was resuspended in 100 ml of ddH_2O, titrated with 0.1 mol L −1 NaOH to pH 11–12. After 4 to 5 h, the suspension was centrifuged at 16,000 r.p.m for 15 min, and insoluble material was discarded. The pH of the supernatant solution, containing insecticidal protein, was adjusted to 4.4 with 25% (v/v^{-1}) acetic acid. The amorphous protein precipitate was separated by centrifugation at 16,000 r.p.m for 15 min, washed repeatedly with ddH_2O and then lyophilized (Zhou *et al.*, 2006). The molecular mass of the preparation, was determined by sodiumdodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Amino acids analysis of purified toxin produced by *Bacillus thuringiensis* var. *kurstaki*:-

The amino acid composition was carried out in The National Research Centre, performed by an amino acid analyzer, type LC 300 – Eppendrof, Germany.

Adsorption and binding studies of purified toxin produced by *Btk* :

The purified toxin of *Bacillus thuringiensis* var. *kurstak* was dissolved in 0.1M phosphate buffer (pH 6), and any insoluble material

was discarded after centrifugation at 16,000 r.p.m for 20 min. The protein content of the toxin preparation was determined by the Lowry method (Lowry *et al.*, 1951), using bovine serum albumin as the standard, and adjusted to the desired concentrations with the buffer. The toxin was added to suspensions of montmorillonite, kaolinite, geothite, silicon dioxide or the clay, and the mineral-toxin mixtures were rotated at 40 r.p.m on a motorized wheel at 24 to 28°C for 3 h, the mixtures were centrifuged at 16,000 r.p.m, and the concentration of the toxins in the supernatants was determined by the Lowry method. The difference between the amount of toxin added and the amount of toxin detected in the supernatants was used to calculate the amount of toxin adsorbed at equilibrium on the clays. Bound complexes were prepared by sequentially washing the adsorbed complexes with dd-H_2O (pH 5.8), with centrifugation at 16,000 r.p.m, until no more toxin was desorbed. The supernatants were analyzed after each wash for the presence of protein. The amount of the toxin bound on the clays was calculated by subtracting the total of the amount of the toxin detected in the equilibrium supernatant and in all washes from the amount of toxin added (Tapp *et al.*, 1994; Tapp and Stotzky 1995 & Venkateswerlu and Stotzky, 1992).

Histopathological studies on larvae of the cotton leaf-worm (*Spodoptera littoralis*):

An assay was performed using cell death detection of 4th instar larvae of cotton leaf-worm (*Spodoptera littoralis*) according to the manufacturer's protocol. After 4 days of feeding of larvea on caster oil leaves treated with purified toxin produced by *Bacillus thuringiensis* var *kurstaki*, the dead larvae were fixed for about 24 h in 10% formaline. The fixed organs were dehydrated and cleared using alchol as a dehydrator and xylene as clearing agent. Embedding in paraffin wax with melting point 55-56°C was followed. Sections were cut at 2µ and the prepared sections were stained by using eosin-haematoxylene (Drury and Wallington, 1980). The stained sections were critically examined and some of the examined parts were photographed at different magnifications. Scoping slides were achieved by using light microscope of the Faculty of Science, Zagazig University, Egypt.

Antifungal activity of toxin produced by *Btk*:

The assays were carried out at the Microbiology Laboratory, where the antifungical activity of Cry1Ab and Cry1Ac proteins of *B. thuringiensis* var. *kurstaki* toxin was examined for the previously mentioned some fungi were supplied from Boteny department, Faculty of

Science, Zagazig University, Egypt (*Trichoderma harzianum, Fusarium oxysporum, Alternaria alternata, Aspergillus flavus, Penicillum sp., Aspergillus fumigatus Fresinus* and *Aspergillus candidus Link & Candida albicans)* was determined by paper disk diffusion. Plates of PDA (Potato Dextrose Agar) medium were inoculated with each fungus and incubated of *Btk* for 7 days. After pape disk saturated with the toxin was placed in the middle of each plate and zone of inhibition was measured (Knaak *et al.*, 2007).

Statistical analysis:

The data were subjected to proper statistcal analysis of variance according to Snedecor and Cochran (1980) and means separation were done according to Duncan (1958).

RESULTS

Purification of toxin produced by *Bacillus thuringiensis* var. *kurstaki*:

The optimization production of bacterial toxin protein as well as biomass by experimental bacterium (*Bacillus thuringiensis* var. *kurstaki*) to obtain high amounts of toxin protein was previously carried (unpublished data). Thus, in the present part of investigation, purification of the crude toxin preparations was carried out. The study included : washing the spray-dried powder by double distilled water for three time, then, removing crystals and spores by cenrifugation (16.000 r.p.m for 15 min.) followed by titration of suspension with 0.1 mol L^{-1}NaOH to pH 11-12. Finally, the suspension was centrifuged at 16.000 r.p.m for 15 min to obtain pure toxin protein.

1. SDS-PAGE protein electrophoresis:

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to a modified method of Laemmli (1970) at the Zagazig University, Faculty of Agriculture. This method was used to determine the molecular weight of the bacterial toxin under study.

Photo (1) show that, lane-2 represents the purified toxin resulted after purification steps, where one band with molecular weight 116 kDa appeared, lane-1 represents the marker (standard) proteins.

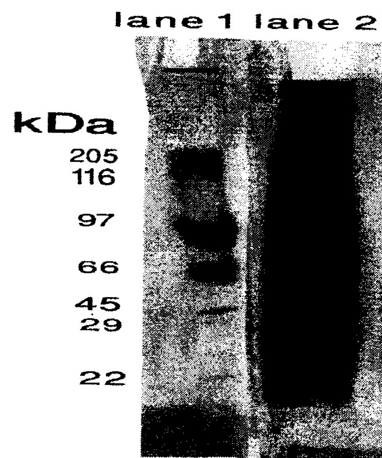

Photo (1): Gel electrophoresis for protien banding.
Lane-1 = *Btk* protein marker.
Lane-2 = *Btk* purified toxin .

2. Amino acids analysis of purified toxin protein:

The amino acid composition of the purified toxin hydrolysate obtained from *Bacillus thuringiensis* var. *kurstaki* was investigated by the authorities of the Central Scientific Services Laboratory of the National Research Centre, Giza, Egypt.

Results recorded in Table (1) and represented graphically in Figs. 1& 2, indicate the presence of 16 amino acids. Proline represented the highest concentration (1299.31567µg/ml), followed by glutamic acid (358.75µg/ml), and cystine represented the lowest concentration (0.58µg/ml) in protein sample. These amino acids, in terms of µg amino acid/ml, were as following; Monoamino- monocarboxylic acids (glycine, alanine, leucine, valine and isoleucine) with the mean concentration of 154.99 µg/ml; Acidic amino acids (glutamic acid and aspartic acid) with the mean concentration of 268.78 µg/ml; Basic amino acids (histidine and lysine) with the mean concentration of 101.83 µg/ml; Oxy- amino acids (serine, threonine and proline) with the mean concentration of 504.91µg/ml, Aromatic amino acids (phenyl alanine and tryosine) with the mean concentration of 160.58 µg/ml and sulfur containing amino acids (cystine, methionine) with the mean concentration of 42.68 µg/ml. While NH^{+4}represented 211.82 µg/ml.

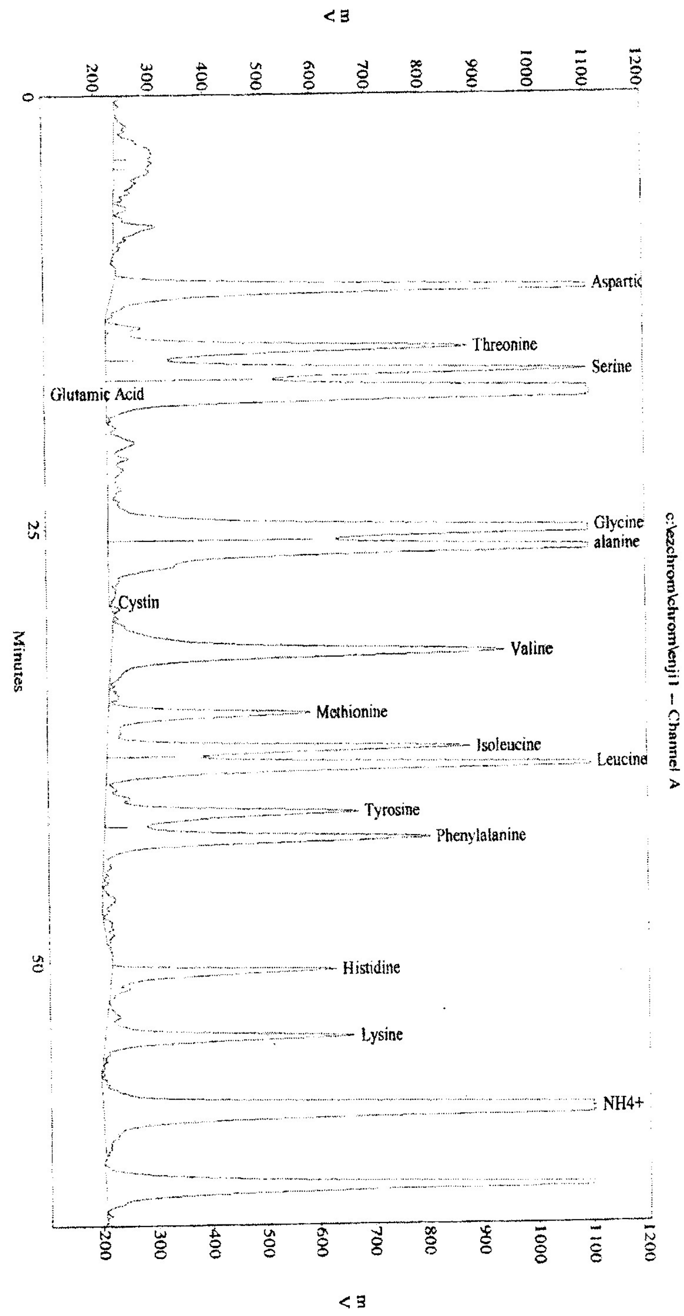

Fig. 1: Amino acid composition of purified toxin produced from *Bacillus thuringiensis* var. *kurstaki*.

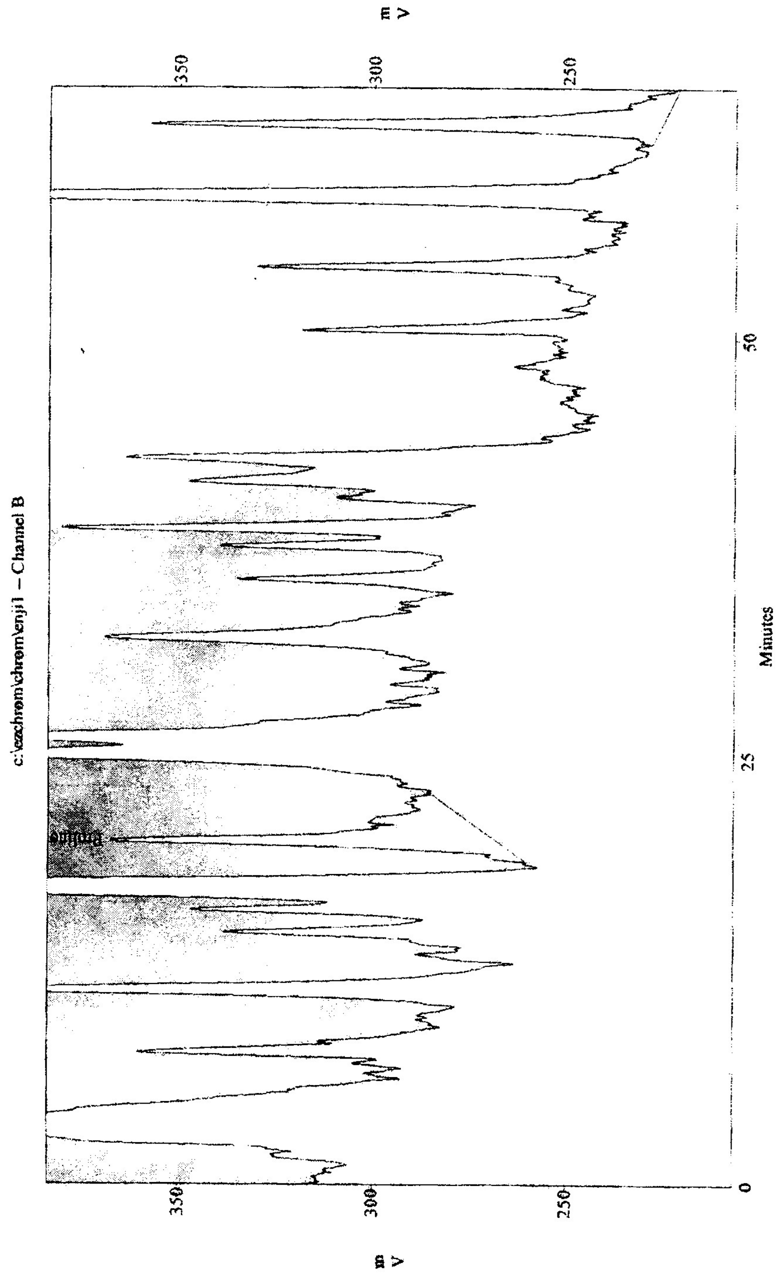

Fig. 2: The highest amino acid composition of purified toxin produced from *Bacillus thuringiensis* var. *kurstaki*.

Table 1: Amino acids content of purified toxin protein produced from *Bacillus thuringiensis* var. *kurstaki*:

pK no	Amino acids	Retentative time (min.)	Area	Amino acid Conc. (μg/ml)
1	Proline	20.08j	6755304	1299.31567a
15	Aspartic	11.13m	36041576	178.80c
18	Threonine	14.67l	24509752	103.01ef
19	Serine	15.98kl	32361170	112.42ef
20	Glutamic acid	17.22k	58650760	358.75b
29	Glycine	25.03i	49087208	81.42f
30	Alanine	26.15i	45408548	143.74de
34	Cystin	29.25h	51874	0.58g
37	Valine	32.20g	35407968	125.80ef
40	Methionine	35.68f	11670734	84.77f
42	Isoleucine	37.68ef	20986414	114.34ef
43	Leucine	38.63e	34326232	194.67c
46	Tyrosine	41.43d	17414362	145.19de
47	Phenylalanine	42.97d	23454472	175.96cd
59	Histidine	50.55c	114202465	107.30ef
62	Lysine	54.33b	15127048	96.35f
68	NH^{+4}	58.48a	55096888	211.82c

Values having the same alphabatical letter(s) did not significantly differ at the 0.05 level of significance, according to Duncan's multiple range test.

Adsorption and binding of purifed toxin protein *Btk* on different minerals:

The aim of this experiment was to investigate the behavior of purified *Btk* toxin in the soil environment.

Five different soils such as montmorillonite, kaolinite, geothite, silicon dioxide and clay were dissolved in 0.1M phosphate buffer (pH6) before and after using (desorption). Results in Table (2) indicate that the amount of soluble protein (24.50μg/ml) was found in 0.15gm of purified toxin (without mineral) produced from *Btk*. The maximum adsorbed

amount of soluble protein of purified *Btk* toxin was achieved with kaolinite (6.69µg/ml) followed by montmorillonite (6.02 µg/ml). The lowest adsorbed amount of protein was recorded in silicon dioxide (5.08µg/ml).

After treatment with phosphate buffer, the toxin adsorbed by different clays was able to desorb to obtain the purified toxin again.

Table 2: Adsorption and binding of protein of purifed toxin from *Bacillus thuringiensis* var. *kurstaki* on different minerals:

Mineral (0.5g/100ml)	Soluble protein (µg/ml)
Control (pure toxin)	24.50a
Geothite	5.76b
Kaolinite	6.69b
Montmorillonite	6.02b
Silicon dioxide	5.08b
Clay	5.60b

Control = protein of purifed toxin without any mineral.

24.50µg/ml of protien was found in 0.15g of purified toxin produced from *Bacillus thuringiensis* var. *kurstaki.*

Values having the same alphabatical letter(s) did not significantly differ at the 0.05 level of significance, according to Duncan's multiple range test.

Histopathological studies:

Mid-gut:

Results represented in Table (3) and figs. 3,4a&4b indicate that *Bacillus thuringiensis* var. *kurstaki* induced severe histopathological effects on the mid-gut. The mid-gut epithelial cells in most areas appeared to be compeletely disorganized, cell boundaries disappeared and slight vacuolation was appearent in some cases. In addition, the basement membrane and surrounding muscles appeared to be detected from the epithelium in some areas. The peritrophic membrane was also destroyed.

Results indicate that all treated larvae with pure *Btk* toxin were dead completely after 4 days in comparsion to control.

Table 3: Effect of pure toxin produced from *Btk* on mortility of larvae at different periods:

Larvae	Mortality (%) of different contact periods (days)			
	1	2	3	4
Control	0	0	0	0
Sample	20	30	70	100

$$\% \text{ of mortility} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

Fig. 3: Section of mid-gut of larvae of cotton warm without *Btk* toxin.

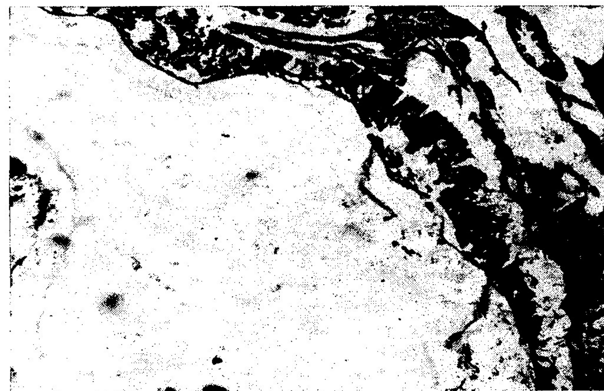

Fig. 4a: Section of mid-gut of larvae of cotton warm with *Btk* toxin.

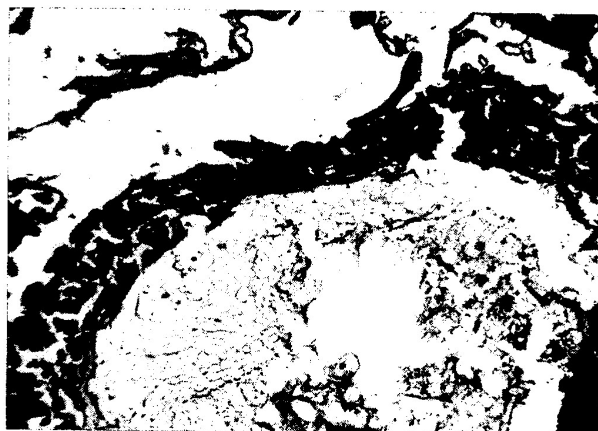

Fig. 4b: Another section of mid-gut of larvae of cotton warm with *Btk* toxin.

Antifungal activity of toxin produced by *Bacillus thuringiensis* var. *kurstaki* :

The results represented in Table (4) reveal that, in general, the tested toxin had a significant antifungal activity against many tested fungal species. *Fusarium oxysporum* was the more sensitive species to *Btk* toxin, while *Candida albicans* was the most resistant one .

Table 4: Antifungal activity of toxin produced by *Bacillus thuringiensis* var. *kurstaki*:

No.	Tested fungus	Inhibition zones(mm)
1	*Trichoderma harzianum.*	14
2	*Fusarium oxysporum.*	30
3	*Alternaria alternata.*	5
4	*Aspergillus flavus.*	10
5	*Penicillum sp.*	7
6	*Candida albicans.*	0
7	*Aspergillus fumigatus Fresinus.*	17.5
8	*Aspergillus candidus Link.*	16.5

DISCUSSION

The molecular mass of the pure toxin preparation, determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), was about 116 kDa. Sleim (2007) reported that the total cellular proteins of the sporulated cells were separated on denaturated gel by electrophoresis (SDS-PAGE) and the protein bands were visualized by staining with Coomassie blue- GR250. The results showed that the protein banding patterns of the group A (Six *Bacillus thuringiensis* bacterial isolates at sporulated growth, showed characteristic protein bands at molecular mass ranging from about 135 to 65 kilo Dalton. Other results recorded that molecular mass protein of *Btk* toxin on T3 medium was 130 kDa (Mohammed, 2006).

The amino acid composition of the purified *Btk* toxin hydrolysate was carried out. Our results recorded that the amino acid analysis showed that the presence of 16 amino acids. Proline represents the highest concentration in protein sample, represents (1299.31567μg/ml), followed by glutamic acid, represents (358.75μg/ml). Other investigators reported that, amino acid composition of δ-endotoxin of *Bt* determines the final structure of a protein, closely related proteins, Cry1Aa and Cry3A, with 36% amino acid sequence identity showed superimposable structure with similar mode of action, whereas Cyt2A protein, which shares less than 20% amino acid sequence identity, is made of single domain with different functional properties (Schnepf *et al.,* 1998). In contrast, several workers reported a single isoleucine N-terminus of pure *Btk* toxin was detected, and the first 20 amino acids were found to be identical with those predicted from the gene nucleotide sequence. A single lysine C-terminus was detected, and the amino acid composition was in excellent agreement with tryptic cleavages at arginine-28 and lysine-623 of the protoxin, and amino acid analysis of the trypsin-insoluble material showed a high lysine and cysteine content and is therefore composed primarily of the C-terminal portion of the protoxin (Bietliot *et al.,* 1989).

One of the most important data concerning the purified *Btk* toxin under investigation that the protein content could be adsorbed on different minerals such as montmorillonite, kaolinite, geothite, silicon dioxide and clay. However, the adsorbed amount by kaolinite was highest at 6.69 μg/ml, followed by montmorillonite (6.02 μg/ml), geothite (5.76 μg/ml), clay (5.60 μg/ml) and silicon dioxide was the lowest (5.08 μg/ml). After treatment with phosphate buffer, the toxin adsorbed by different clays was able to desorb to obtain the purified toxin again. In inspection of findings

of other investigators, adsorption was higher with montmorillonite than with kaolinite or illite and depended on the nature of the compensating cation (Stotzky, 2000). Parallel results were recorded by Yong *et al.* (2008). Fu *et al.* (2007) measured the desorption of bound toxin by NaCl and phosphate buffer. Low concentrations (<10 mmol L^{-1}) of organic acid anions inhibited toxin adsorption by kaolinite, goethite, and silicon dioxide, whereas high concentrations promoted adsorption.

The experimented purified toxin was evaluated against newly moulted 4th instar larvae of cotton leaf-worm (*Spodoptera littoralis*). *Bacillus thuringiensis* var. *kurstaki* induced severe histopathologies effects on the mid-gut. The mid-gut epithelial cells in most areas appeared to be compeletely disorganized, cell boundaries disappeared and slight vacuolation was appearent in some cases. In addition, the basement membrane and surrounding muscles appeared to be deteched from the epithelium in some areas. The peritrophic membrane is also destroyed. The same observations were previously obtained by Labib and Dawoud (2003), Gamil (2004) and Nasr (2005) who studied the effect of the entomopathogens *Bacillus thuringiensis* var. *kurstaki* on the mid-gut of the 4th instar larvae of *Spodoptera littoralis* and observed the disruption of the columner epithelium cells and detachement and destruction of the basement membrane and peritrophic membrane and vacuolization and destruction of the epithelial cells. Other investigators reported that, the bioinsecticide *Bacillus thuringiensis* var. *kurstaki* (*Btk*) was used for controlling the mosquito species (*Anoppheles stephensi* and *Culex quinquefasciatus*) which gave a significant ($p<0.05$) mortality in both species (Kumar *et al.*, 2009).

Many investigators studied the impact of bacterial toxins in insects. These toxins of *Bacillus thuringiensis* are responsible for the inhibition of protein synthesis by forming a protein complex. The recorded data indicated that, *B. thuringiensis* resulted in a great reduction in protein content of 4 instar larvae of *S. littoralis*. These th data confirm the findings of Angus and Norvis (1968) who demonstrated that d-endotoxin of *B. thuringiensis* forms a complex protein having an alkaline isoelectric pH. Also, Lecadet and Martouret found that the crystal (Lecadet and Martouret, 1967a,b) like endotoxin of *B. thuringiensis* splits into protein and peptide. Obvious reduction in haemolymph protein concentration of 3 instar *S. littoralis* larvae treated with *B. thuringiensis* var. *kurstaki* (*Btk*) combined with some chemical additives was found by Latha *et al.*, (1996).

Concerning the antifungal activity of the *Btk* toxin against tested fungal species, the results revealed that, in general, the tested toxin had an antifungal activity against some tested fungal species. The larger inhibition zone occurred with *Fusarium oxysporum* and reached 30 mm. This result was inconformity with the results of serveral workers, where *Bacillus thuringiensis* strains showed positive effects on phytopathogenic fungi. This result was in conformity with the results of several workers, where, proteins synthesized by *Bacillus thuringiensis thuringiensis* and *B. thuringiensis kurstaki* were assessed in the following phytopathogens: *Rhizoctonia solani*, *Pyricularia grisea*, *Fusarium oxysporum* and *F. solani*, which had their myicelial growth decreased after incubation in the presence of the bacterial strains. As to Cry proteins, there were no inhibition halo development in the assessed concentrations (Knaak *et al.*, 2007). It can be seen that the inhibiting effect of *B. thuringiensis* strains on phytopathogenic fungi breed can be associated with enzyme production that can act against the fungal cell wall, since some bacteria antagonistic of phytopathogenic fungi bring about chitinases (Mavingui and Heulin, (1994) & Asaka and Shoda, (1996)).

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دراسة تنقيه وادمصاص السم البروتيني البكتيري المنتج من بكتيريا الباسيليس سيرينجينسيس كيوريستاكي

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هدفت هذه الدراسة إلى تنقية السم البروتيني البكتيري المنتج من بكتيريا الباسيليس سيرينجينسيس كيوريستاكي وذلك عن طريق تحضير كميه كبيره من الراشح البكتيري، ثم تجفيف هذا الراشح للحصول عليه في صورة (مركزه) ثم يتم غسل هذه البودره ثلاث مرات بواسطة ماء مقطر مزدوج . يتم بعد ذلك عمل الطرد المركزي عند ١٦،٠٠٠ الفه في الدقيقه لمدة ١٥ دقيقه وذلك لفصل الجراثيم المنقاه من الراشح. يتم معايرة المحلول باستخدام هيدروكسيد الصوديوم ٠,١ مول (عند اس ايدروجيني ١١-١٢). و يتم طرده مركزيا لمدة ٤/١ ساعه عند سرعة ١٦،٠٠٠ الفه في الدقيقه لنحصل على السم البروتيني البكتيري .

بتحليل السم البكتيري المنقى وجد ان الوزن الجزئي لهذا السم حوالي ١١٦ كيلودالتون باستخدام طريقة الهجره الكهربائيه للرقائق المعلقه على البولي اكريلاميد.

وبتحليل الأحماض الامينيه للسم البروتيني البكتيري وجد انه يحتوي على ١٦ نوع من الأحماض الامينيه أكثرهم تركيزا هو البرولين حيث يمثل١٢٩٩,٣١٥٦٧ ميلليميكرون/ ميلليميتر يليه حمض الجيليوتاميك حيث يمثل ٣٥٨,٧٥ميلليميكرون/ ميلليميتر من المحتوى البر وتيني الكلي من العينة.

وبدراسة تأثير ادمصاص السم البكتيري على انواع مختلفه من التربه الطينيه. بعض المعادن المستخلصة من التربة مثل جيوثيت (أكسيد الحديد المائي)، كاولينيت ، مونتمورونيت ، أكسيد السيليكون والطمي إلي السم المنقى وجد أن مونتموريلونيت هو أكثر المعادن قدره على امتصاص السم البر وتيني يليه كاولينيت.

تم دراسة تأثير السم البروتيني البكتيري المنقى على يرقة دوده ورقة القطن في الطور الرابع من العمر حيث وجد ان اليرقات ماتت جميعا في اليوم الرابع مقارنه بالغير معامل.ولقد اوضح التشريح الهيستولوجي ان خلايا الجهاز الهضمي لليرقه المعامله بالسم البكتيري المنقى قد تأثرت تأثيرا ملحوظا مقارنة بالعينه الضابطه حيث اظهر الفحص الميكروسكوبي تحلل الخلايا المبطنه الجهاز الهضمي مما يشير الى اعراض تسمم ملحوظه بهذا السم البروتيني. واظهرت النتائج ان للسم البروتيني البكتيري نشاط ضد فطري ضد الفطريات مثل أسبرجلس فلافس، ترايكودرما فيريدى، فيوزاريوم أوكسيسبورم، ألترناريا ألترناتا، كانديدا ألبيكانس،بينيسيليم اسبيشيس، أسبرجلس فيوميجاتس فرنسينس، أسبرجلس كنديدس لنك، ووجد ان فطر فيوزاريوم أوكسيسبورم اكثر الفطريات تأثر للسم البكتيري بينما فطر كانديدا ألبيكانس لم يتأثر للسم البكتيرى.