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MOLTEN GLOBULE STRUCTURE OF *BACILLUS THURINGIENSIS V*AR. *KURUSTAKI* INSECTICIDAL PROTEIN COULD BE NECESSARY TO INDUCE HIGHER MORTALITY RATE IN SPODOPTERA *LITTORALIS*.

BY

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ABSTRACT

The role of molten globule structure of *Bacillus thuringiensis* var. *kurustaki* (*B.t.*) in inducing the insecticidal activity against *Spodoptera littoralis* was studied throughout the present investigation. The obtained data showed that kosmotropic (kosmo-trope=order maker) materials like sucrose and glycerol could increase *B.t.* Cry proteins toxicity mostly through their stabilizing function for proteins. Chaotropic cosolvents (chao-tropic=order-breaking) such as, urea at low concentrations, resulted an increase in *B.t.* Cry proteins toxicity supposed through their destabilizing native protein structures. Meanwhile the kosmotropic material, ethylene glycol, the good stabilizer ammonium sulfate and wheat flour (as a source of protein with disulfide bonds) exhibited reduction in *B.t.* Cry protein activity. Also, addition of boiled water to the Cry proteins decreased toxicity rates. On the contrary, addition of boiled water to the Cry proteins mixed with wheat flour resulted in toxin preparation, which gave 100% mortality to the 2nd instar larvae of *Spodoptera littoralis* (Boisd.) after 2 days of treatment, that represented 650% increase in *B.t.* activity. This would suggest that Cry proteins toxicity might decrease at both, very high stabilizing and destabilizing environment while marginal increase in toxicity was obtained at marginal increase or decrease in stabilization of Cry protein structures. It is suggested that toxicity induced by Cry proteins structures resulted from incubating Cry proteins with wheat flour (which contains proteins with disulfide bonds) with boiled water for one hour suggest that Cry proteins partially unfolding by thermal denaturation in the presence of protein with cross linkers could induce partly ordered forms which exhibit high content of secondary structure, considerable compactness, nonspecific tertiary structure, and structural flexibility indicating that molten globule structure of *Bacillus thuringiensis* var. *kurustaki* insecticidal protein is necessary to induce sufficient mortality rate in *Spodoptera littoralis* (Boisd.)

INTRODUCTION

A crucial factor in protein product development is preservation of functional peptide structures. To retain the function of proteins, the peptide structure has to be folded in the unique native conformation, which is only marginal stable and very sensitive towards external factors e.g. temperature, pH, and chemical composition of the solvent. Therefore these molecules often have a tendency to denature under various production, storage and application conditions. The requirement of industrial protein to function under severe conditions is high and thus highlights the need for rational formulation strategies. (Bagger, 2007). *Bacillus thuringensis* bacteria produce insecticidal proteins (Cry) toxic to different insect species and nematodes (Schnepf, *et al.* 1998). The primary action of Cry toxin is to lyse midgut epithelial cells in the target insect by forming lytic pores on the apical membrane (Schnepf, *et al.,* 1998). Kosmotropic (kosmo-trope=order maker) materials have

been used in the present study for their stabilizing function for proteins. On the other hand the insecticidal crystal (Cry) proteins produced by *Bacillus thuringiensis* undergo several conformational changes from crystal inclusion protoxins to membrane-inserted channels in the midgut epithelial cells of the target insect (Rausell, *et al.*, 2004a). For this reason, chaotropic agents (chaotropic=order-breaking) at low concentrations have been used in the present study to achieve partial unfolding and flexibility of the toxin structure.

The main focus in the present study is enhancement activity of *B.t.* Cry protein at concentration (2.5 g/l) used in all trials, which correspond to about the same concentration of *B.t.* used in field application.

MATERIAL AND METHODS

1.Laboratory rearing of tested *Spodoptera littoralis* (Boisd.) larvae:

One colony of *Spodoptera lottoralis* was established from egg masses obtained from a continuous laboratory culture maintained since long time at the Plant Protection Research Institute, Dokki, Cairo, Egypt. The larvae of *S. littorais* were maintained in the laboratory on castor bean leaves. Emerging moths were provided with 10% honey solution as a food . Adults were allowed to mate and oviposit their eggs on paper sheets. After eggs hatching, the larvae were fed on fresh castor bean leaves.

2. *Bacillus thuringiensis* subspecies *kurustaki* (*B.t.*):

Commercial name: Dipel-2x

Active ingredient:6.4% ***Bacillus thuringiensis***

Formulation: Wettable Powder (32000IU/G).

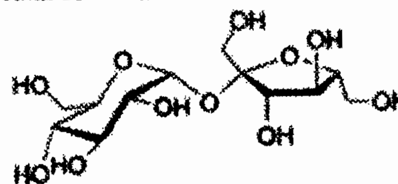
Source :Abbot Laboratories.

Structure of B. thuringiensis δ-endotoxin. (A) Schematic ribbon representations of the CryIIIA toxin showing the domain organization as determined by Li *et al.,* 1991: domain I (colored), the pore-forming domain; domain II (gray), the receptorbinding domain; and domain III (black). The helices of the pore-forming domain are colored in a rainbow direction: α1, red; α2, orange; α3, yellow; α4, green; α5, cyan; α6, blue; and α7, purple. These illustrations were made by using the rasmol program. (B) Sequences of the pore-forming helices and their corresponding synthetic peptides. (Gazit, *et al.,* 1998)

3.Additives materials used:

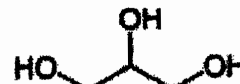
1- Sucrose:

Sucrose (common name: Table sugar, also called saccharose) is a disaccharide of glucose and fructose, with the molecular formula C12H22O11



2- Glycerol:

Glycerol is a chemical compound also commonly called glycerin or glycerine.

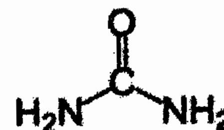


3- Ethylene glycol 99%:

Ethylene glycol (monoethylene glycol (MEG), 1,2-ethanediol, is an alcohol with two -OH groups (a diol).

4- Urea B.p 93:

Urea is an organic compound with the chemical formula (NH2)2CO.

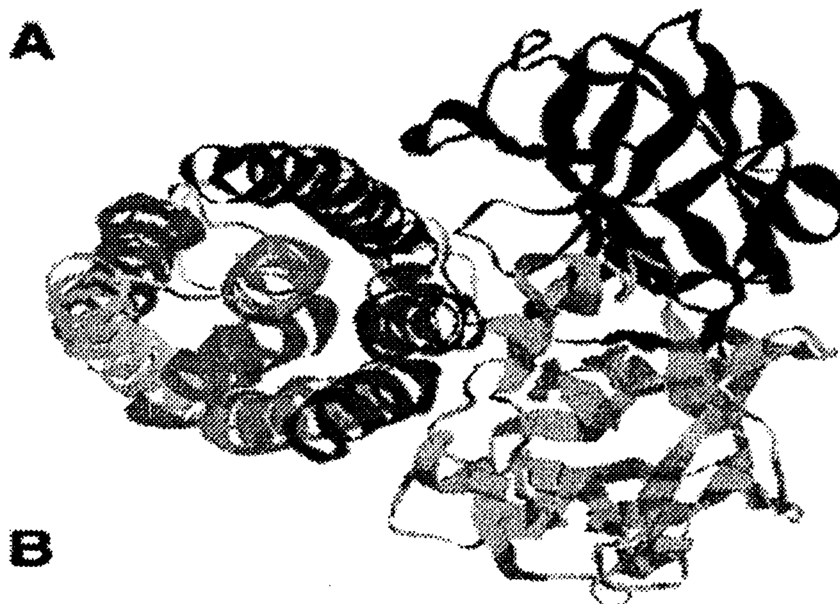


5- Boiled water:

Boiled tap water.

6-Wheat flour:

Bread flour, (high in gluten,) with a certain toughness that holds its shape well once baked. Gluten is a composite of the proteins *gliadin* and *glutenin*. These exist, conjoined with starch in the endosperms of some grass-grains, related notably wheat, rye, and barley

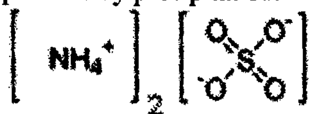


α1 X-NH-KDVIQKGISVVGDLLGV-cooH
α2 X-NH-GGALVSFYTNFLNTIWPSEDPWKAFMENVEALM-cooh
α3 X-NH-DYAKNKALAELQGLQNNVEDYVSALSSWQK-cooH.
α4 X-NH-HSQGRIRELFSQAESHFRNSMPSTA-cooH.
α5 X-NH-FLTTYAQAANTHLFLLKDAQKYG-cooH.
Z
α6 X-NH-KEDIAEFYKRQLKLTQEYTDHCVKWYNVGLDKL-cooH.
α7 X-NH-YESWVNFNRYRREMTLTVLDLIALF-cooH.

Fig. (A): **Struture of *Bacillus thuringiensis Cry proteins.* (Picture adapted from Gazit, *et al.*, 1998)**

7- **Ammonium sulfate** (pure lab. Chemicals):

Ammonium sulfate, (NH4)2SO4, is an inorganic chemical compound commonly used as a fertilizer. In biochemistry, ammoium sulfate precipitation is a common method for purifying proteins by precipitation.



4.Bioassay:

1. **Insecticidal activities of tested additives materials with Dipel-2x:**

The dipping technique was followed to test the activities of Cry toxin alone and combined with additives. Homogeneous pieces of castor bean leaf were dipped into suspensions for 10 seconds and shade dried before offering to 2 hours starved 2nd instar larvae. Only one single concentration of Dipel-2x was used (2.5g/l) which almost corresponds the same rate for field application. Toxicity of this concentration was taken as a base line for comparative toxicities between treatments used throughout the investigation. Different concentrations for each additive material were mixed with the recommended concentration of *B.t.* (Dipel-2x). The healthy second instar larvae of *S. littoralis* were used in bioassay. Each concentration was replicated 3 times with 10 larvae each. Larvae were fed on treated food for 2days after which fresh untreated leaves were offered. Larval mortalities were recorded after 2,4 and 7days of feeding on treated castor bean leaves. Mortality data were corrected for natural mortality according to Abbot's equation (Abbot, 1925). The % increase or decrease in mortality rates were calculated according to the equation:

2- **Role of tested additives materials on Dipel-2x insecticidal activity in the presence of activation energy:**

These experiments were carried out by using dipping technique. The *B.t.*. powder

(0.625g) with additive concentrations were mixed together in a glass bottles. Boiled water was added to dilute up to 250 ml. The suspensions were left for one hour for the heat to be turned down to the room temperature

RESULTS AND DISCUSSION

Part 1: Effect of kosmotropic and chaotropic materials on *B.t.* Cry protein activity:

The kosmotropic materials used were sucrose, glycerol and ethylene glycol. The tested concentrations were 5, 10, 15, 20 and 40 g (ml)/l. Data in Tables 1-3 and Fig. 1-3 indicated that sucrose and glycerol kosmotropic materials increased the activity of *B.t.* Cry protein (Dipel-2x) at 2.5 g/l. Sucrose (20g/l) increased mortality by 73% more than efficacy of Dipel alone after 2days post treatment, while glycerol achieved 67% increase by 40ml/l after the same period. By elongation of feeding period to 4 and 7 days, sucrose (40g/l) increased activity of *B.t.* by 32% and 13% respectively, while the corresponding increase by glycerol (20ml/l) reached 27% and 10% respectively. All concentrations of ethylene glycol showed reduction in *B.t.* activity for all counting.

Sucrose and glycerol were used to stabilize biological systems, whereas urea and guanidine hydrochloride were used to solubilize coagulated systems and to unfold (denature) proteins. These additives, were directed to function as cosolvents, and to displace to the right or left the chemical equilibrium, Reactant □ Product (Timasheff 2002).

The mechanism of stabilization of invertase with polyols (glycerol, xylitol, and sorbitol) was studied by Gangadhara and Prakash (2008). They showed that polyols act as very effective stabilizing agents.

Urea as a chaotropic material was used at 9 low concentrations to partially unfold and increase the activity *of B.t.* Cry protein against the 2nd instar larvae of *S. littoralis* (Table 4 and Fig. 4). Almost all concentrations used increased *B.t.* activity. Highest increases in mortality rates was recorded: (300%) at (0.24g/l) after 2days post treatment, (188% and 44%) after 4 and 7days respectively, at (0.2g/l) .

The stability of monomeric, pre-pore and pore structures of Cry1Ab toxin after urea and thermal denaturation suggested that a more flexible conformation could be necessary for membrane insertion and this flexible structure is obtained by toxin oligomerization and by alkaline pH. Domain I is involved in the intermolecular interaction within the oligomeric Cry1Ab and this domain is inserted into the membrane in the membrane-inserted state (Pardo-López *et al* 2006).

Moelbert *et al* (2004) reported that chaotropic cosolvents, such as urea destabilizes hydrophobic aggregates, micelles and native protein structures.

Part 2: Effect of salt with Hofmeister behaviour on *B.t.* activity:

Recent investigations in concern of ion-specific effects are traced back to a series of studies by Hofmeister and his co-workers published at 1880s and the 1890s. They classified salts according to their ability to "salt-in" or "salt-out" proteins from solution. "Hofmeister series" as shown in Fig.(B) is generally presented as a cat-/anion-specific ranking in relation to some measured response. (Evens and Nieds 2008).

A salt from pairs of the first ions in these series (for example, $(NH_4)_2SO_4$), when added to aqueous solutions of proteins, precipitate the native form of the protein. The protein is precipitated in the native, not denatured state. The last ions of the series destabilize the native state . (Dill 1990).

Data in (Table 5 and Fig. 5) indicate that ammonium sulfate which is a strongly stabilizing material for proteins induced great reduction in *B.t.* activity at all concentrations tested 20, 40, 80, 120 and 160 g/l.

Table (1): % increase or decrease in mortality rates in relation to Dipel 2x alone treatment

Dipel-2x (2.5g/l) with sucrose conc. (g/L)	Days after treatment		
	2 Days	4 Days	7 Days
Di alone	0	0	0
Di + 5 g. suc.	-13	-27	-33
Di + 10 g. suc.	13	-9	-21
Di + 20 g. suc.	73	27	8
Di + 40 g. suc.	53	32	13
Di + 80 g. suc.	0	18	0

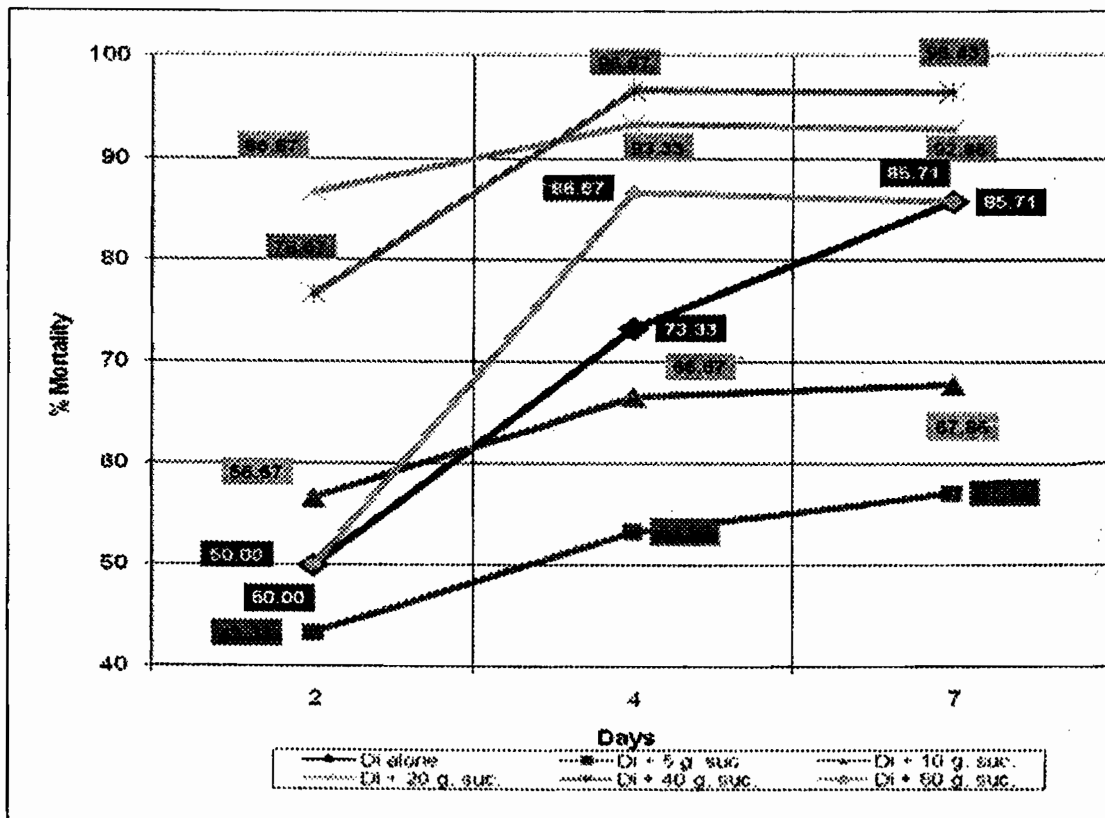


Fig. (1): Mortality percentages after different periods of feeding *S. littoralis* 2nd instar larvae on castor bean leaves treated with Dipel-2x (with and without sucrose concentrations)

Values inside rectangles represent mortality percentages

Table (2): % increase or decrease in mortality rates in relation to Dipel-2x alone treatment.

Dipel-2x (2.5g/l) with glycerol conc. (ml/L)	Days after treatment		
	2 Days	4 Days	7 Days
Di alone	0	0	0
Di + 5ml.gly.	7	-14	-5
Di + 10ml.gly.	20	14	0
Di + 20ml.gly.	40	27	10
Di + 40ml.gly.	67	27	10
Di + 80ml.gly.	13	14	0

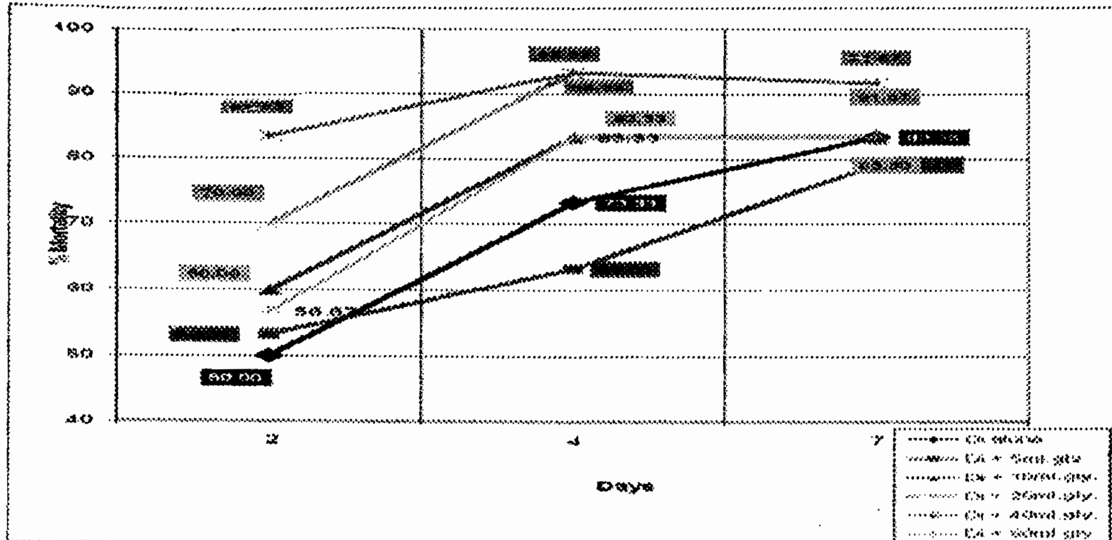


Fig. (2): Mortality percentages after different periods of feeding *S. littoralis* 2nd instar larvae on castor bean leaves treated with Dipel-2x (with and without glycerol concentrations)

Table (3): % increase or decrease in mortality rates in relation to Dipel 2x alone treatment

Dipel 2x +ethylene glycol conc.(ml/l)	Days after treatment		
	2 Days	4 Days	7 Days
Di alone	0	0	0
Di.+5ml.ethylene gl.	-80	-76	-44
Di.+10ml.ethylene gl.	-93	-90	-50
Di.+20ml.ethylene gl.	-93	-76	-47
Di.+40ml.ethylene gl.	-93	-76	-47
Di.+80ml.ethylene gl.	-33	-57	-32

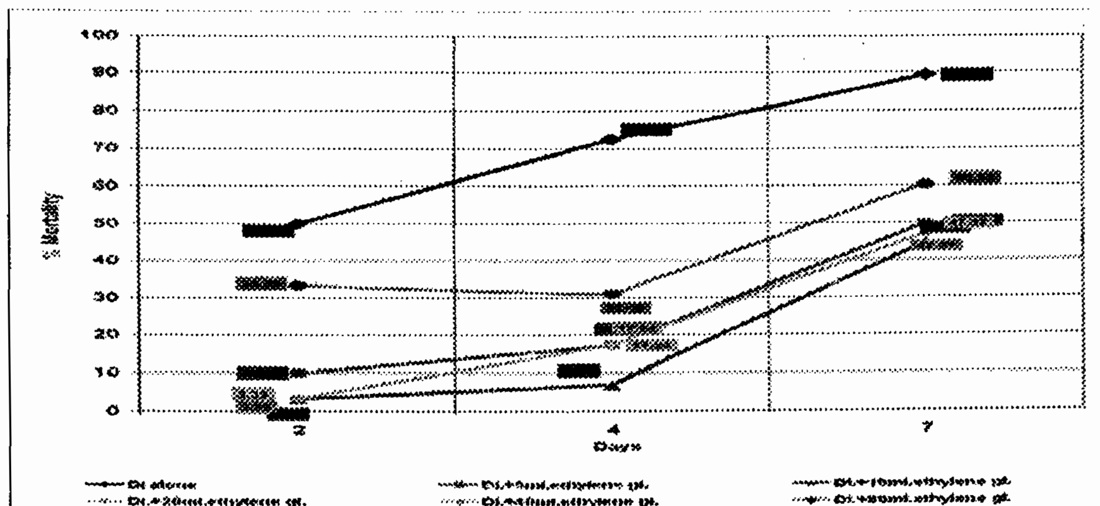


Fig. (3): Mortality percentages after different periods of feeding *S. littoralis* 2nd instar larvae on castor bean leaves treated with Dipel-2x (with and without ethylene glycol concentrations)

Values inside rectangles represent mortality percentages

Table (4): % increase or decrease in mortality rates in relation to Dipel-2x alone treatment

Dipel 2x(2.5g/l) with urea conc. (g/l)	Days after treatment		
	2 Days	4 Days	7 Days
Di alone	0	0	0
Di.+0.08g.urea	67	63	-6
Di.+0.12g.urea	233	138	25
Di.+0.16g.urea	67	25	-19
Di.+0.2g.urea	267	188	44
Di.+0.24g.urea	300	100	25
Di.+0.28g.urea	267	175	38
Di.+0.32g.urea	-33	-38	-19
Di.+0.36g.urea	133	0	-25
Di.+0.4g.urea	200	50	0

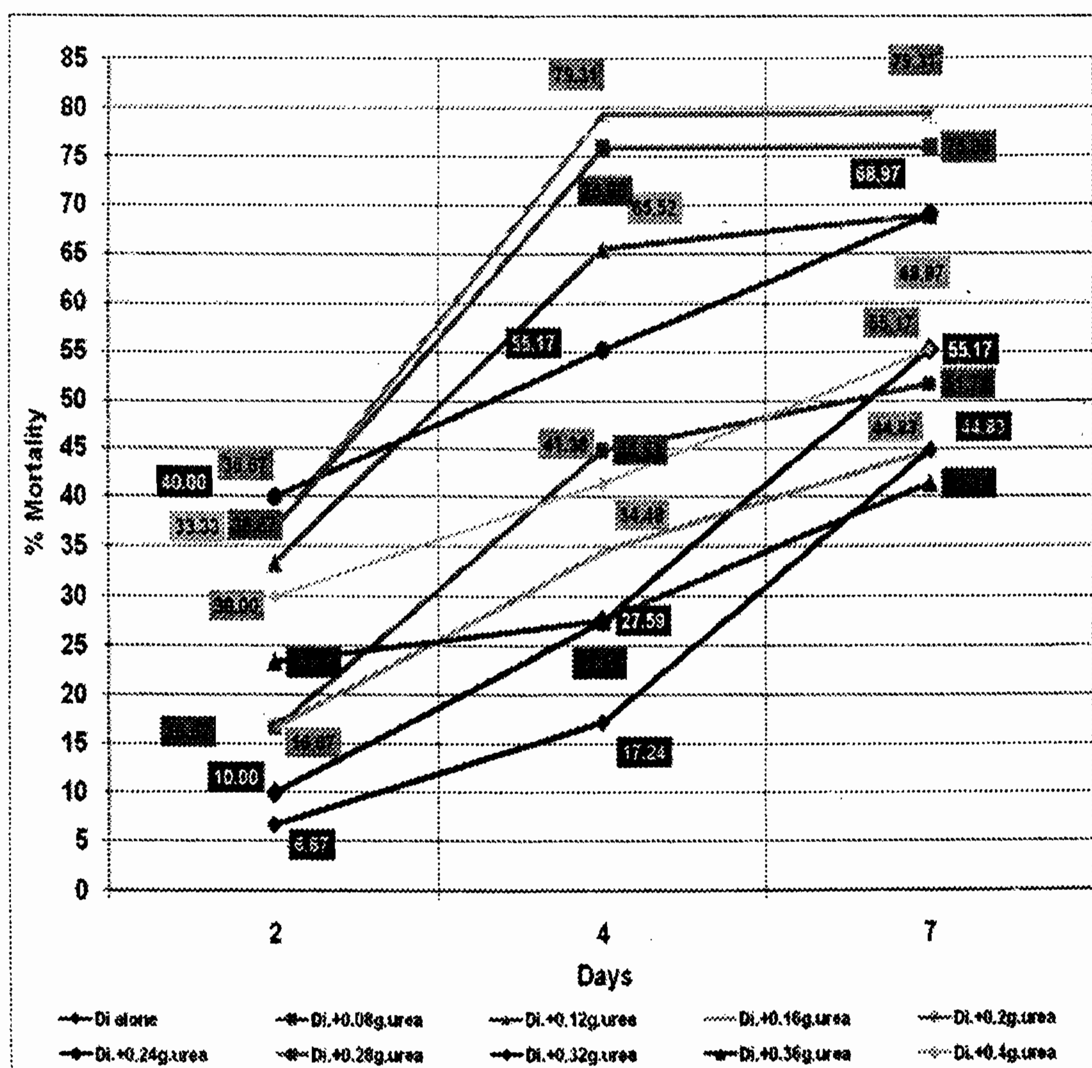


Fig. (4): Mortality percentages after different periods of feeding *S. littoralis* 2nd instar larvae on castor bean leaves treated with Dipel-2x (with and without Urea concentrations)

Values inside rectangles represent mortality percentages

Table (5): % increase or decrease in mortality rates in relation to Dipel-2x alone treatment

Dipel-2x(2.5g/l) with ammonium sulfate conc. (g/l)	Days after treatment		
	2 Days	4 Days	7 Days
Di alone	0	0	0
Di+20g.ammo.sulfate.	-100	-95	-54
Di+40g.ammo.sulfate.	-87	-73	-73
Di+80g.ammo.sulfate.	-67	-68	-73
Di+120g.ammo.sulfate.	-93	-82	-77
Di+160g.ammo.sulfate.	-80	-77	-62

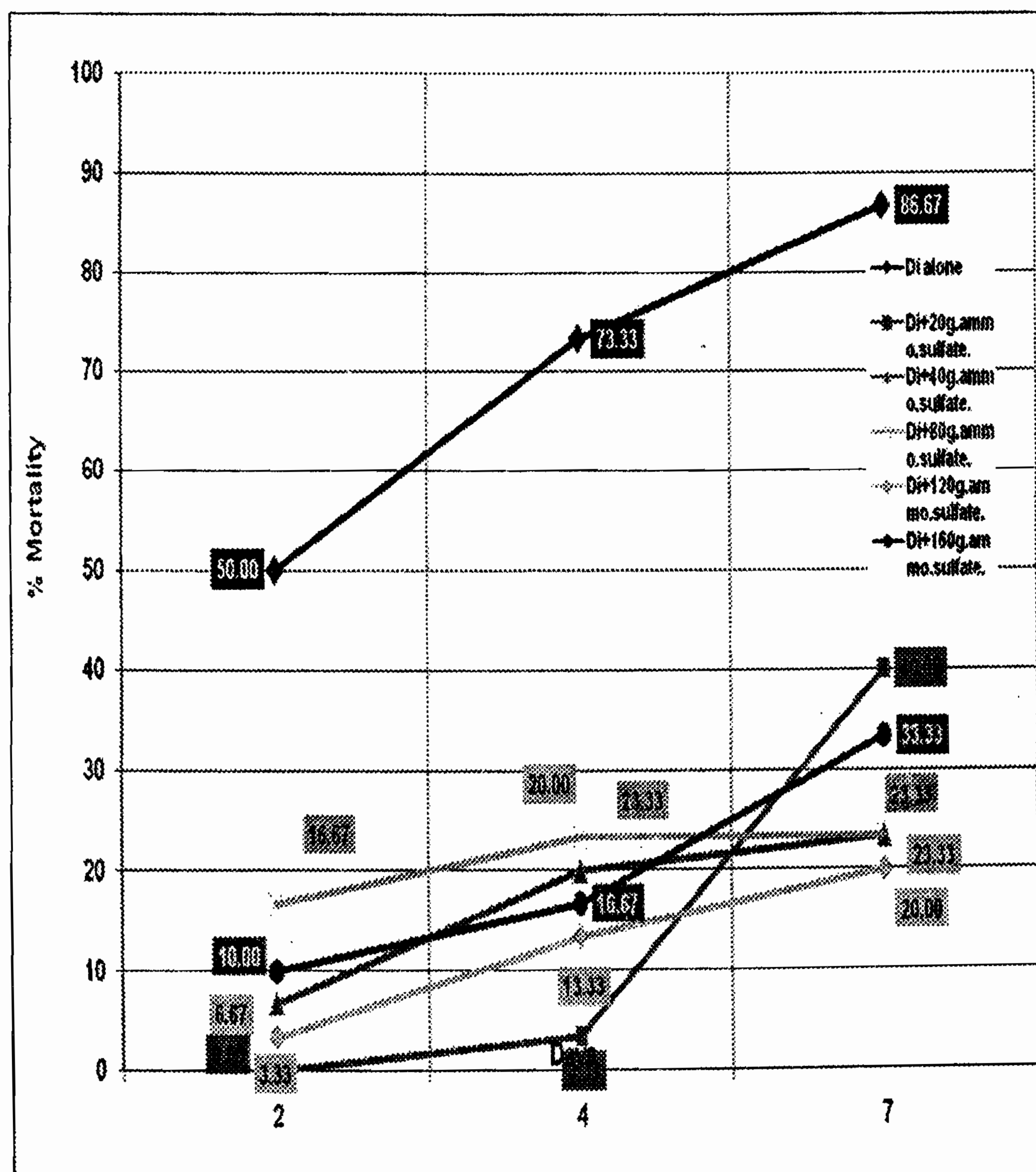


Fig. (5): Mortality percentages after different periods of feeding *S. littoralis* 2nd instar larvae on castor bean leaves treated with Dipel-2x (with and without ammonium sulfate concentrations)

Values inside rectangles represent mortality percentages

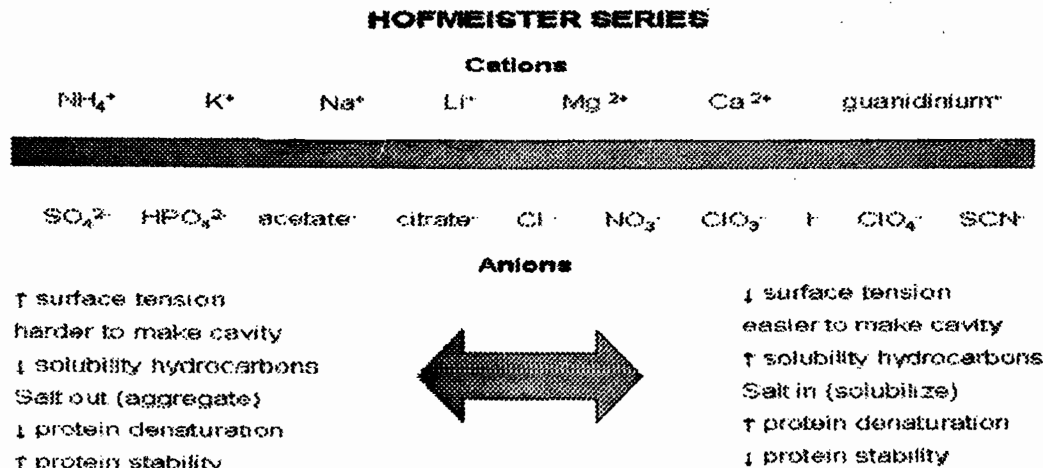


Fig.(B) "Hofmeister series"

Part 3: Effect of protein cross-linkers on *B.t.* activity:

Wheat flour as a source of gliadin and glutenin proteins with disulfide bonds was added to *B.t.* Cry protein (Dipel 2x, 2.5g/l) at 40, 20, 10, 5, and 2.5 g/l and bioassayed on the 2nd instar larvae of *S.littoralis*. Data in Table (6), illustrated in Fig.(6) show that all wheat flour rates used reduced *B.t.* activity i.e. mortality percentages were lower than those obtained by Dipel-2x treatment alone. For example (40g/l) conc. induced reduction in *B.t.* activity by 25%, 23, and 0% after 2, 4 and 7 days of treatment respectively. That wheat flour conc. was used in a separate trial with activation energy (for comparison). Interestingly that wheat flour (40g/l) in the presence of activation energy (boiled water) increased *B.t.* activity by 650% than *B.t.* treatment alone after 2 days post treatment.

In proteins that contain cysteine residues, the folding of side chains may bring these groups into proximity. Their oxidation to yield disulfide crosslinks adds greatly to the stability of the protein molecules. Protein molecules may contain crosslink of non-covalent, eg. salt bridges or a covalent, eg. disulfide bonds Mangino and Harper (2007). Mariel *et al.* (2002) stated that rational design of mutants in order to increase the stability of a protein essentially aims to either improving stabilizing interactions or reducing potentially destabilizing factors. Because of its large stabilizing potential and relatively well defined conformational requirements, the introduction of disulfide bonds is an attractive method for improving stability, but this strategy has been applied with mixed success. Although the introduction of disulfide cross-links theoretically should have a stabilizing effect, the opposite is often observed. This destabilization can be attributed to negative effect on packing and flexibility. This may be the reason of decreased efficacy of *B.t.* Cry proteins after mixing Dipel-2x with wheat flour which contains proteins with disulfide bonds.

Part 4: The contribution of water and activation energy in *B.t.* activity:

The interactions of water with proteins are very important both to the structure and function of the proteins. (Mangino and Harper 2007).

In order to study the contribution of water on *B.t.* Cry protein activity, (2.5 g.) of Dipel-2x was suspended in 250 ml water. There was a trial of dipping castor bean leaves in *B.t.* suspension every 1 hr. for bioassay on the 2nd instar larvae of *S. littoralis* (Table 7 and Fig. 7). Data showed differences in % mortality obtained by the same concentration of Dipel-2x, at different periods of incubating *B.t.* Cry protein in water during the period of trial.

Boiled water (as a source of increased temprature) was added to *B.t.* Cry protein (Dipel-2x) (10g/l). The experiments were performed one hr. later after suspensions temperature was turned down to room

temperature. There was an experiment every one hr. up to 6 hrs. Data in Table (8) and illustrated in Fig.(8) showed that there are fluctuations in *B.t.* Cry protein activity in solution from time to time. The activity of Dipel-2x (10g/l) became lower in the presence of increased temperature.

The results in part 1, 2 and 3 may indicate that the stabilizing effect of the kosmotropic materials could increase the *B.t.* Cry protein activity, but increasing *B.t.* Cry protein stabilization too much (by good stabilizer or by introducing disulfide bonds) could decrease *B.t.* Cry protein toxicity. On the other hand the, destabilizing effect of chaotropic material is needed for *B.t.* Cry protein partially unfolding needed for membrane insertion and pore formation. This can be explained by the more stability nature of the *B.t.* Cry protein (Dipel-2x) induced by the strongly stabilizing agents (ethylene glycol, ammonium sulfate and protein with disulfide bonds in wheat flour) compared with the native *B.t.* Cry protein which makes it harder for α4-loop-α5 hairpin to insert into the membrane. In contrast, the chaotropic material, urea at low concentrations can increase the activity rates of *B.t.* Cry protein (Dipel 2x). This is because the *B.t.* Cry proteins after interaction with cadherin receptor undergo conformational changes from a monomeric structure to a pre-pore-oligomeric form that is able to interact with a second GPI-anchored aminopeptidase-N receptor and then insert into lipid membranes. (Pardo-López,*et al.*, 2006). According to the umbrella model (Li, *et al.*, 1991, Gazit, *et al.*, 1998 and Masson, *et al.*, 1999.), domain I binds the membrane. After the initial binding to the receptor, the protein undergoes a transformation in which structural rearrangement occurs. Presumably, this is followed by a hydrophobic α4-loop-α5 hairpin insertion into the phospholipid bilayer, whereas the amphipathic helices are spread on the surface. Later, oligomerization occurs, and a channel or pore is formed.

Part 5: The introduction of molten globule structure necessary for pore forming:

Wheat flour as a source of gliadin and glutenin proteins with disulfide bonds was added to *B.t.* Cry protein (Dipel-2x, 2.5g/l) at concentrations 40, 20, 10, 5, and 2.5 g/l and bioassayed on the 2nd instar larvae of *S. littoralis*. Data in (Table,6) show that all wheat flour concentrations used reduced *B.t.* activity.

Data shown in Table (9) demonstrate the effect of activation energy (as boiled water) when added to (Dipel-2x)(2.5g/l) + Wheat flour at 40, 20, 10, 5, and 2.5 g/l. As illustrated in Fig.(9) there were a great deal of % increase of *B.t.* activity against 2nd instar larvae of *S.littoralis* obtained with two wheat flour rates only 40g/l and 20g/l. Wheat flour at (40g/l) in the presence of activation energy induced 650%, 107% and 115% increase in *B.t.* activity after 2, 4 and 7 days post treatments respectively. Wheat flour at (20g/l) with activation energy induced 275%, 79% and 79% increase in *B.t.* activity after 2, 4 and 7 days, respectively. On the contrary all remained wheat flour rates (10g/l, 5g/l and 2.5g/l) caused different trends of reduction in *B.t.* activity. This could be attributed to the partially unfolding of insecticidal crystal proteins which could be induced by thermal denaturation with boiled water and the presence of wheat flour which contains gliadin and glutenin proteins with its disulfide inter- and intra-molecule links increases the stability through a 3-dimensional network that forms when sulfur cross-linkages develop between protein molecules. After that, molten globule state could be induced and that is necessary for membrane insertion. This explanation could be in agreement with Pardo-Lopez *et al.* (2006), they reported that analysis of the stability of monomeric, pre-pore and pore structures of Cry1Ab toxin after urea and thermal denaturation suggested that a more flexible conformation could be necessary for membrane insertion and this flexible structure is obtained by toxin oligomerization and by alkalin pH. According to Gomez *et al.*, 2002; Rausell *et al.*, 2004b, and Muñoz-Garay *et al.*, 2006), the formation of Cry oligomeric structures has been demonstrated for Cry1Aa, Cry1Ab, Cry1Ca, Cry1Da, Cry1Ea, Cry1Fa and Cry3 toxins. In all cases, the Cry toxin samples containing oligomeric structures correlated with high pore activity, in contrast to monomeric samples that showed marginal pore-formation activity, supporting the hypothesis that oligomer formation is a necessary step in the mechanism of action of Cry toxins.

Table (6): % increase or decrease in mortality rates in relation to Dipel-2x alone treatment

Dipel-2x(2.5g/l)+flour conc. (g/l)	Relative toxicity of tested mixtures		
	2 Days	4 Days	7 Days
Di alone	0	0	0
Di.+40g.flour	-25	-23	0
Di.+20g.flour	-75	-69	-54
Di.+10g.flour	-75	-54	-23
Di.+5g.flour	-100	-100	-85
Di.+2.5g.flour	75	-38	-31
40 g flour + A. Energy	650	115	115

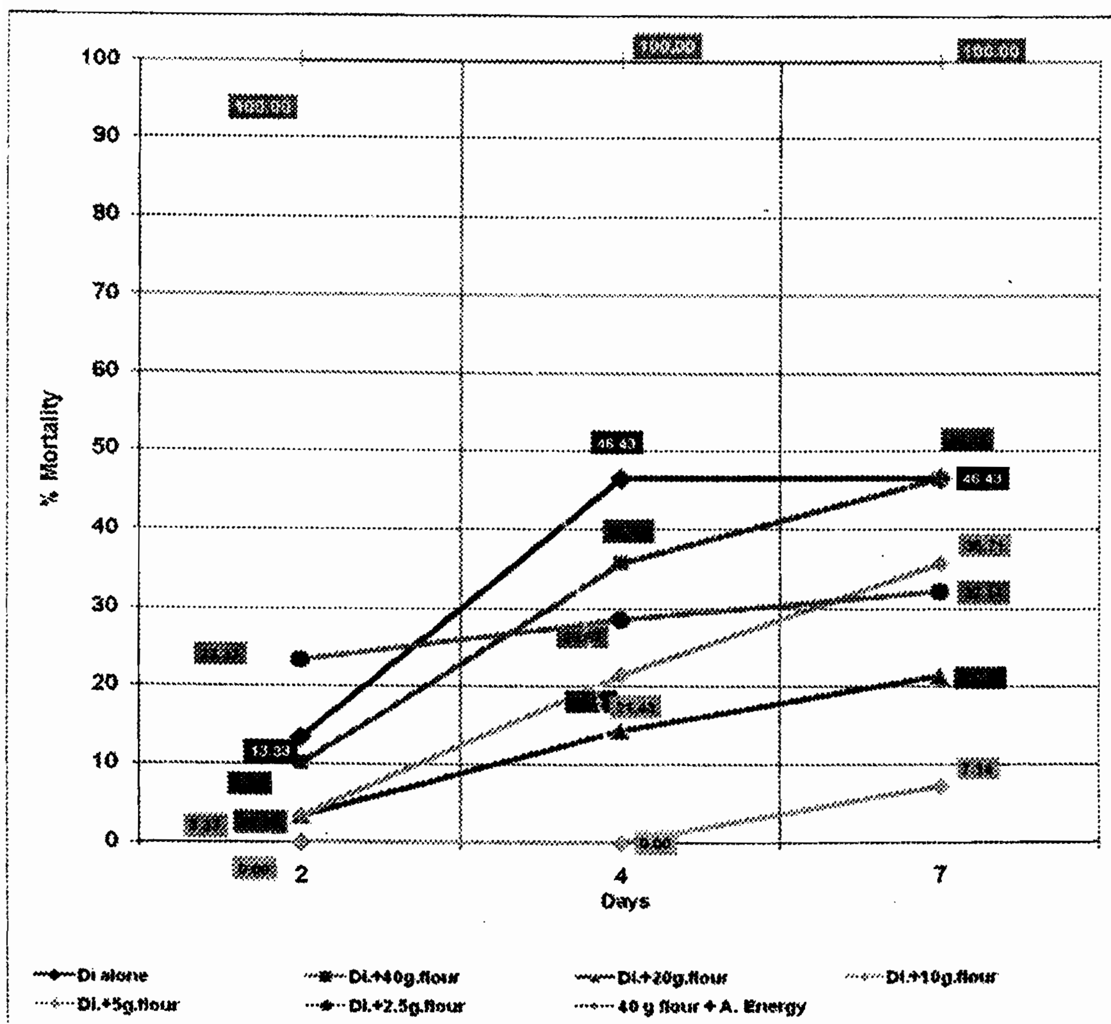


Fig. (6): Mortality percentages after different periods of feeding *S. littoralis* 2nd instar larvae on castor bean leaves treated with Dipel-2x (with and without wheat flour concentrations). Activation energy was added with the 40g conc. only for comparison

Values inside rectangles represent mortality percentages

Table (7): The contribution of water in protein denaturation (or protein activity) corresponding to the effect of incubation period (0-6 hours and 7days) of Cry. protein Dipel-2x (10 g/l) in water on the activity on the 2nd instar larvae of *S.littoralis*

incubation periods	Corrected Mortality % ± SD			Average
	2 Days	4 Days	7 Days	
Zero time	33.33± 15.28	62.07± 34.82	62.96± 41.67	**52.79**
1 hr.	73.33± 05.77	86.21± 12.24	92.59± 06.61	**84.04**
2 hrs.	30.00± 26.46	51.72± 39.91	48.15± 38.95	**43.29**
3 hrs.	83.33± 15.28	93.10± 06.12	92.59± 06.61	**89.68**
4 hrs.	23.33± 25.17	37.93± 35.42	40.74± 29.50	**34.00**
5 hrs.	40.00± 26.46	58.62± 36.72	59.26± 37.35	**52.63**
6 hrs.	66.67± 32.15	86.21± 12.24	85.19± 08.36	**79.35**
7 hrs.	50.00± 26.46	68.97± 34.32	70.37± 32.02	**63.11**
8 hrs.	33.33± 15.28	62.07± 34.82	62.96± 41.67	**52.79**
9 hrs.	50.00± 26.46	58.62± 36.72	55.56± 13.44	**54.73**
10 hrs.	70.00± 26.46	86.21± 12.24	88.89± 17.32	**81.70**
11 hrs.	0.00± 00.00	72.41± 24.48	70.37± 32.02	**47.59**
12 hrs.	46.67± 15.28	68.97± 34.32	77.78± 22.28	**64.47**
13 hrs.	0.00± 00.00	62.07± 34.82	66.67± 33.39	**42.91**
7 days	10.00± 00.00	10.34± 10.00	7.41± 06.12	**9.25**

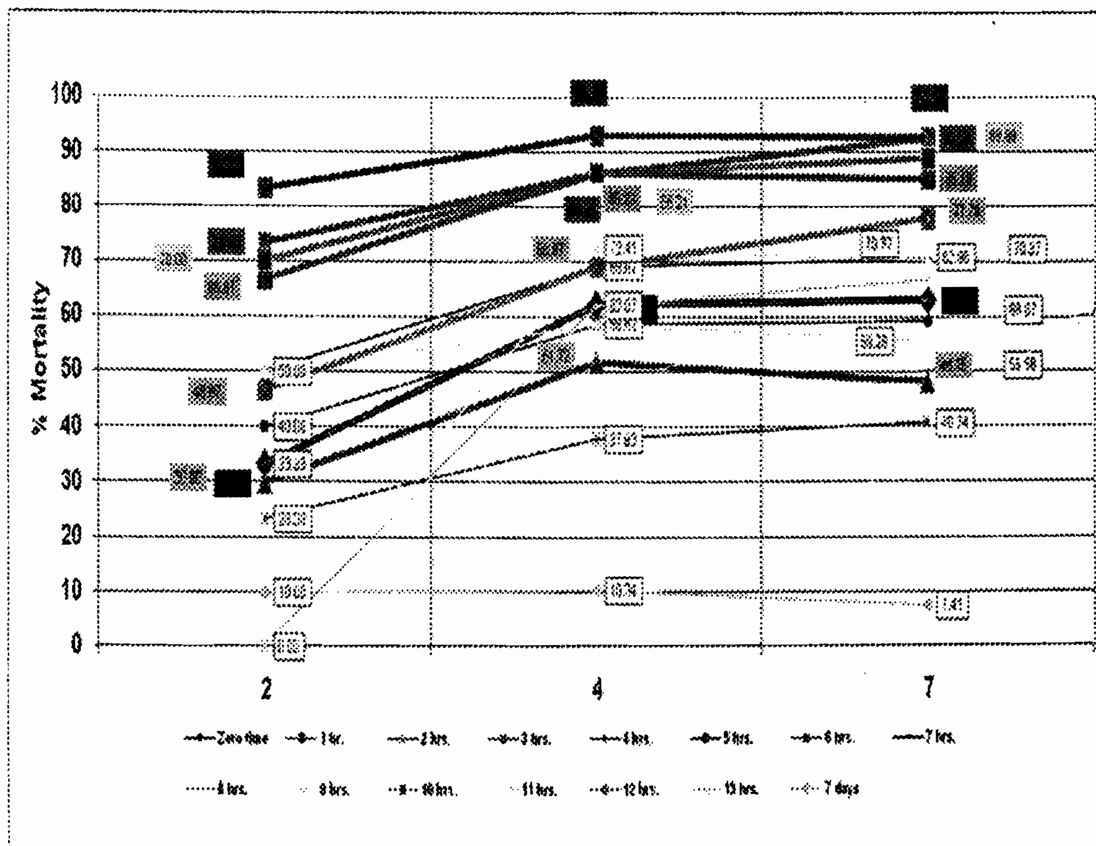


Fig. (7): Mortality percentages after different periods of feeding *S.littoralis* 2nd instar larvae on castor bean leaves treated with Dipel-2x (10g/l) (in normal water)(at different incubation periods of *B.t.* Cry. Protein).

Values inside rectangles represent mortality percentages

Table (8): Effect of the activity of Dipel-2x(10 g/l) alone in boiled water (the suspension was left to turned down to room temperature before testing) on the 2nd instar larvae of S.*littoralis* at 1-6 hours (and 7 days) solution incubated periods

Incubation perods of the solution	Corrected Mortality % ± SD			Average
	2 Days	4 Days	7 Days	
Zero time	--	--	--	-
1 hr.	33.33± 20.82	51.72± 03.21	53.57± 08.01	46.21
2 hrs.	30.00± 20.00	48.28± 13.47	46.43± 11.56	41.57
3 hrs.	26.67± 11.55	51.72± 03.21	60.71± 24.04	46.37
4 hrs.	56.67± 32.15	58.62± 34.02	64.29± 31.29	59.86
5 hrs.	16.67± 15.28	20.69± 20.00	28.57± 23.52	21.98
6 hrs.	23.33± 25.17	55.17± 24.15	53.57± 08.01	44.03
7 days	6.67± 05.77	27.59± 10.96	25.00± 07.14	19.75

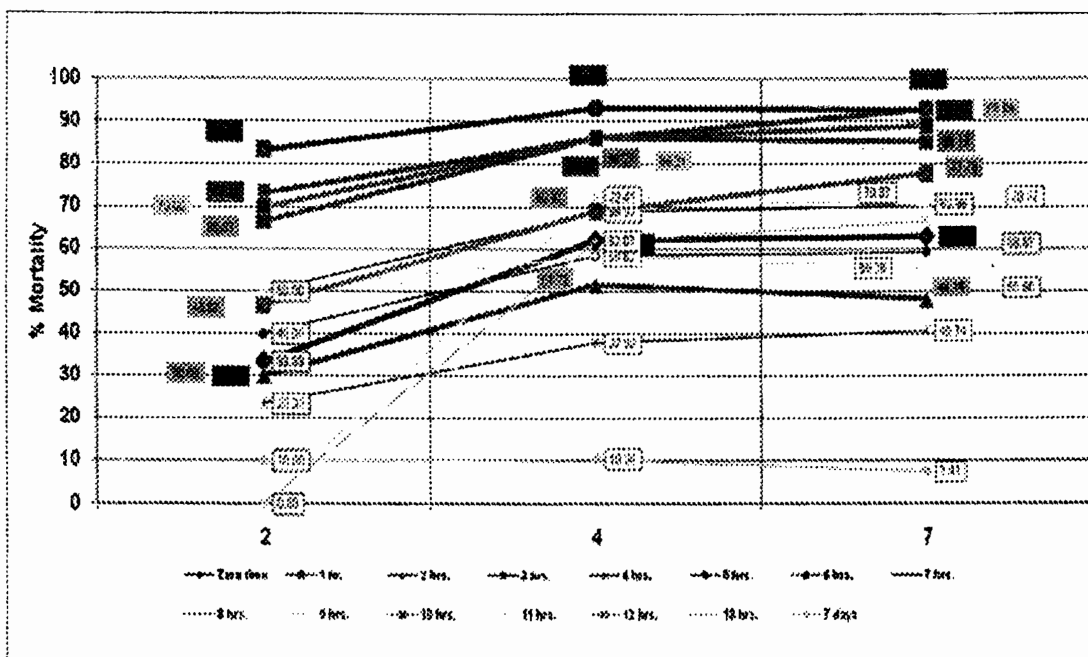


Fig. (8): Mortality percentages, after different periods of feeding *S.littoralis* 2nd instar larvae on castor bean leaves treated with Dipel-2x (10 g/l) alone(in the presence of activation energy) at 1-6 hours (and 7 days) solution incubated periods.
Values inside rectangles represent mortality percentages

Table (9) % increase or decrease in mortality rates in relation to Dipel-2x alone treatment

Dipel 2x + activiation energy + flour conc. (g/l)	Days after treatments		
	2 Days	4 Days	7 Days
Di alone	0	0	0
Di.+40g.flour+act.energy	650	107	115
Di.+20g.flour+act.energy	275	79	100
Di.+10g.flour+act.energy	-50	-64	-46
Di.+5g.flour+act.energy	0	-79	-77
Di.+2.5g.flour+act.energy	0	-50	-46

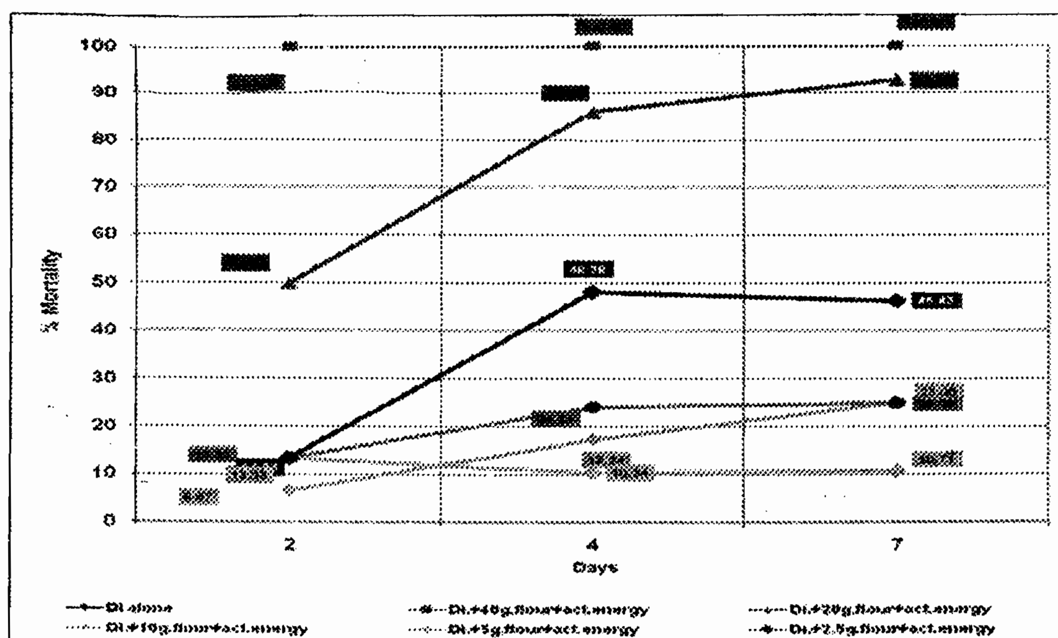


Fig. (9): Mortality percentages after different periods of feeding *S. littoralis* 2nd instar larvae on castor bean leaves treated with Dipel-2x (with and without wheat flour concentrations in the presence of activation energ).

Values inside rectangles represent mortality percentages

CONCLUSION

As conclusion, the kosmotropic mater-ials sucrose, and glycerol can increase the activity rates of *B.t.* Cry protein (Dipel-2x). In addition, the chaotropic material, urea at low concentrations can increase the activity rates of *B.t.* Cry protein (Dipel-2x) against the 2nd instar larvae of *S.littoralis*. Meanwhile the kosmotropic materials, ethylene glycol, the good stabilizer ammonium sulfate and wheat flour (as a source of protein with disulfide bonds) exhibited reduction in *B.t.* Cry protein activity. Also boiled water when added to *B.t.* Cry protein powder decreased the toxicity. On the contrary, when wheat flour (40g/l) was combined with (Dipel-2x) powder (10g/l) in the presence of activation energy (boiled water), the activity of *B.t.* Cry protein became extremely high so that it caused 100% mor-tality of *S. littoralis* second instar larvae, 2 days after treatment.

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التركيب المسال للبلورات البروتينيه السامه للبكتيريا باسيلوس ثورينجيينسيز ضرورى لزيادة مستوى السميه على يرقات حشرة دود ورق القطن

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تم دراسة الدور الذى يلعبه التركيب المسال للبلورات البروتينيه السامه للبكتيريا باسيلوس ثورينجيينسيز بهدف زيادة مستوى السميه على يرقات حشرة دود ورق القطن وتشير النتائج المتحصل عليها فى هذه الدراسة أن المواد التى تساعد على زيادة ثبات البروتين مثل السكروز - الجليسرول قد ساعدت علي زيادة سمية الكريستال بروتين لبكتيريا باسيلليس ثورينجيينسيس (دايبيل ٢ اكس) على يرقات العمر الثانى لدودة ورق القطن وايضا المواد التى تعمل على عدم ثبات البروتين مثل اليوريا (التركيزات العاليه) عند إستخدامها فى هذه الدراسه بتركيزات منخفضه أدت الى زيادة سمية ال دايبيل ٢ اكس ومن جهه اخرى فإن المواد التى تعمل على زيادة ثبات البروتين بدرجه كبيره مثل سلفات الأمونيوم والإثيليين جليكول بالإضافة الى دقيق القمح بما يحتويه من جلوتين به بروتينات زات محتوى عال من الروابط ثنائية الكبريتيد عند اضافتها الى الكريستال بروتين لبكتيريا باسيلليس ثورينجيينسيس (دايبيل ٢ اكس) أدى ذلك

الى خفض السميه على يرقات العمر الثانى لدودة ورق القطن، وكذلك إنخفضت السميه عند اضافة درجة الحرارة العاليه فى صورة ماء مغلى. وعلى العكس من ذلك فإن اضافة الماء المغلى الى الكريستال بروتين لبكتيريا باسيلليس ثورينجيينسيس (دايبيل ٢ اكس) فى وجود دقيق القمح والتحضيين لمدة ساعه قبل معاملة ورق الخروع بهذا المستحضر وتقديمه كغذاء ليرقات العمر الثانى لدودة ورق القطن ادى ذلك الى موت اليرقات بمقدار ١٠٠% بعد يومين اثنين من المعامله وتمثل هذه النتيجه زيادة فى فاعلية الكريستال بروتين لبكتيريا باسيلليس ثورينجيينسيس (دايبيل ٢ اكس) بمقدار ٦٥٠%. ويمكن أن تفسر هذه النتائج على أن الزيادة الكبيرة فى ثبات الكريستال بروتين لبكتيريا باسيلليس ثورينجيينسيس (دايبيل ٢ اكس) ربما تؤدى الى انغلاق الكريستال بروتين بدرجه كبيره على الجزء المسئول عن السميه وهوالجزء الغير محب للماء ويقع فى مركز الكريستال بروتين وبذلك لا يستطيع التحرر بسهوله داخل معدة الحشرة لاحداث السميه، وعلى العكس من ذلك فان اضافة الماء المغلى الى الكريستال بروتين ربما يحدث عدم ثبات للجزء الخارجى من الكريستال بروتين فى الوقت الذى يظهر فيه مركز الكريستال بروتين وبه الجزء المسئول عن السميه ومع تواجد دقيق القمح بما يحتويه من جلوتين به بروتينات زات محتوى عال من الروابط ثنائية الكبريتيي قد يؤدى ذلك الى ارتباطات جديده وربما زياده كبيره فى ثبات مركز الكريستال بروتين وهذا التركيب المسال للبلورات البروتينيه السامه للبكتيريا باسيلوس ثورينجيينسي قد يوحى بضرورة هذا المكون فى احداث سميه عاليه ليرقات دودة ورق القطن.