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EVALUATION OF *Bacillus thuringinesis* ALONE OR MIXTURE WITH AMINO ACID FOR CONTROLLING COTTON LEAFWORM , *Spodoptera littoralis* (Boisd.)

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ABSTRACT

Second instar larvae of *Spodoptera. littoralis* were fed on caster bean leaves treated with different concentrations of Dipel (*Bacillus thuringiensis* var. *kurstaki*). after 72 hours of treatment (estimated LC_{50}) Regarding the LT_{50} values a negative relationship could be detected between the applied concentration of Dipel and LT_{50} value; i.e. the LT_{50} was shortened with the increase in Dipel concentration. The LT_{50} values being 14.17, 8.81,5.99 and 2.40 days for the used concentrations of 8, 12, 16 and 20 × 10^4 I.U. The second instar *S. littoralis* larvae were fed on fresh caster bean leaves treated with different concentrations of Dipel + 05 % Amino. , the corrected mortality percentages after 3 days increased by increasing concentration ranged from 40.00 to 90.00 % at the concentrations of 8 to 28 × 10^4 I.U., the LC_{50} value was 11.31 × 10^4 I.U. for *S. littoralis* after 3 days., comparing the effects of the two treatments (Dipel and Dipel + 0.5% Amino) on the mortality percentages at same concentration used, Diple + 0.5 % treatment led to increase the percentages of mortality in all concentrations used and thus led to decrease the value of LC_{50} from 17.14 to 11.31 × 10^4 S.U. and shortened also the values of LT_{50} from 14.7, 8.81 and 5.99 to 6.66,3.88 and 2.18 days at concentration of 8, 12 and 16 × 10^4 I.U. , respectively, The LC_{50} of Diple2x + 1% Amino Acid was 15.59× 10^4 I.U. 72 hours post treatment , a negative relationship could be detected between the applied concentration of Dipel + 1% Amino and. the LT_{50} was shortened with the increase in treatments concentration. These values were 11.61, 7.97, 3.99, 3.07 and 1.72 days for the used concentrations of 8, 12, 16, 20 and 24 × 10^4 I.U. , respectively . LC_{50} of Diple2x + 1.5% Amino Acid was 16.06 × 10^4 I.U. after 3 days of treatment. and LT_{50} values. were 14.12, 6.73, 3.13 and 1.69 days at concentrations of 8,12,16 and 20× 10^4 I.U. , respectively.. The LC_{50} of Diple2x + 2.5% Amino Acid was was 16.62 × 10^4 I.U.. After3 days from treatment while the LT_{50} values were 11.18, 6.56, 4.15 and 2.80 days at concentrations of 8, 12, 16 and 20 × 10^4 I.U., respectively. , LC_{50} value of Dipel 2x + 0.5 % amino (11.31× 10^4 I.U.) was the most effective one among all treatments ,also, led to shortened the values of LT_{50} (6.66, 3.88 and 2.18 days for the used concentrations of 8, 12 and 16 × 10^4 I.U. , respectively

Keywords: *Bacillus thuringiensis* var. *kurstaki*, *Spodoptera. Littoralis*, Amino Acids, mixture.

INTRODUCTION

Overuse of Chemical pesticides have caused considerable environmental problems and they have even threatened human health (Gill ***et al.,*** 1992). The bioregional insecticide *Bacillus thuringiensis* is a useful alternative to chemical pesticides that has been developed for the control of

certain insect pests. The biological insecticides based on *B. thuringiensis* have been valued for their environmental safety, their low development costs, and their specific activity against certain insect pests (Lambert and Peferoen, 1992).

Recently, microbial insecticides consider as a component of biological control techniques are developed and encouraged. They give good results against insect pests without polluting the environment (Amer *et al.,* 2012). Besides, giving low toxicity to non-target animals and humans (Aranda *et al.,* 1996). The most abundant and successful microorganism used as effective bioinsecticide was *B. thuringiensis* (Cartton, 1988; De Maagd *et al.,* 2001 and Ibrahim & Omar, 2005).The basis of *B. thuringiensis* insecticidal activity comes from the δ-endotoxin formed during sporulation and is also toxic to insect larvae belong to order Lepidoptera (El- Husseini *et al.,* 2012). *B. thuringiensis* infected larvae showed significant higher percentages of cellular apoptosis at 12 an 24h post-infection compared to control ones. These data may indicate that *B. thuringiensis* infection induced oxidative stress and apoptosis proceeding cellular damage. (Ashraf (2013). Also,, *B. thuringiensis* Induces Cellular Stress in the Mosquito Vector,*Culex pipiens*, Prior to Death.

(Treshow, 1970; El- Naggar, 1998; Mesbah *et al*., 2000; El- Naggar, 2009 and Magdoff *et al.,* 2000) indicated farming practices that cause nutrition in balance can lower pest resistance.

Amino acids compositions in plant sap play critical role in determining susceptibility to sap sucking pests (Tsumuki *et al.,* 1987). Rice plants with less asparagine content show resistance against brown planthopper (Sogawa and Pathak 1970). Resistant lines of oats and barley contain less quantity of asparagine and higher amounts of glutamic acid (Weibul *et al.,*1987). Resistance lines of oats and barley contain less quantity of asparagine and higher amounts of glutamic acid (Weibull *et al.,*1987; Hedin *et al.,* 1990). Aspartic acid metabolic pathway controls the biosynthesis of amino acids asparagine, aspartic acid and glutamic acid in plants. Aspartic acid is the starting compound for two main pathways, one leads to the synthesis of asparagine and other to aspartate-derived amino acids viz., lysine, threonine, methionine and isoleucine (Azevedo *et al.,* 1997). The present study aimed to evaluate the impact of *B. thuringiensis* alone or in mixtures with Amino acids to against cotton leaf worm , *Spodoptera littoralis* .

MATERIALS NAD METHODS

1-Rearing of *S. littoralis*

A laboratory stock culture of *S. littoralis* started with larvae collected from the field and maintained under constant conditions of 27 ±3 °C & 65 ± 5 % R.H. according to the methods recorded by Mansour (2001) .

2-Tested compounds :

a- Dipe 2x 6.4 % a selective bacterial insecticide containing 32×10^6 I.U. of *B. thuringiensis* var. *kurstaki* / gm. of product.

b-Canada Amino: The main components are:Nitrogen 5% -Phosphorous 3% - Potassium 4% -Iron 1.5% - Zinc 1.1% - Manganese 1.1% - Humic acid 10% -amino acids 10% -active fulvic acid 2.5%.

3- Treatments:

Five experimental treatments were carried out as follows:

a- Bioinsecticidal treatments:

Weights of 2.5, 3.75, 5.00 , 6.25 , 7.5 and 8.75 gm. of Dipel 2x were diluted in water to obtain a constant volume of 200 ml (total volume), to represent the concentrations of 8, 12, 16 , 20, 24 and 28 × 10^4 I.U., respectively.

b- Bioinsecticide + different concentrations of Amino Acids:

A volume of 7.5, 15, 22.5, and 37.5 ml of Canada Amino were diluted in water to obtain constant volume of 1.5 liter (total volume) of to give solution of concentrations of 0.5, 1, 1.5 and 2.5 %. For each prepared solution six concentrations of 8, 12, 16 , 20, 24 and 28 × 10^4 I.U. of Dipel 2x were prepared as previously described.

The following procedures were applied:

For each concentration of any tested treatment, three replicates, each of ten second instar larvae, placed in a jar for rearing to feed on the caster bean leaves treated with the bioinsecticide or with bioinsecticide + different concentrations of amino. Mortality rates were recorded daily. Larvae that survived after treatment were transferred to other jars containing untreated caster bean leaves. Before exposing the larvae to treated food, they were starved for 4 hours in order to obtain rapid simultaneous ingestion of the contaminated food.Control test was conducted by dipping clean caster bean leaves in water, left to dry and then offered to the experimental larvae.The experiments were carried out under laboratory conditions of 27 ± 3 °C and 65 ± 5 % R.H. The castor-bean leaves were dipped for one minute in each of the used concentrations, and then treated leaves were left for air dryness and offered to the tested larvae.

Statistical analysis:

The effectiveness of the different treatments were expressed in term of LC_{50} values at 95 fiducially limits slopes of regression lines were represented. Statistical analysis of the obtained data was made based on the analysis of variance and liner regression analysis (Finney, 1971 and slide write program). In addition, polynomial regression procedure in COSTAT program was done.

RESULTS AND DISCUSSION

I- Bioinsecticide (Dipel 2x) treatments:

The daily corrected mortality percentages resulting from the treatment of 2nd instar larvae of *S. littoralis* are shown in Table (1). Larval mortality percentages, 30 days post treatment were 63.33, 73.33, 76.67, 80.00, 83.00and 83.33 %at concentrations of 8, 12, 16, 20, 24 and 28 × 10^4 I.U.., respectively. The percentages mortality after 72 hours of treatment (estimated LC_{50}) ranged from 36.67 to 63.33 % at concentrations of 8 × 10^4 to 28× 10^4 I.U (fig 1) It is evident from Table (1) that the percentages mortality

increased by increasing the concentration. The LC_{50} value was 17. 4 × 10^4 I.U. 72 hours post treatment (Table, 2 and Fig., 1). These results are in agreement with

The increased mortality percentages by increasing the concentrations of Dipel 2x agree with those previously reported by: Kares *et al.,* (1992) on larvae of the cabbage worm *Artogeia rapae* when testeing Bactospeine; Badawy (2000) when he tested Dipel 2x, Ecotech bio and MVP_{11} against *S. littoralis* and the potato tuber moth *Phthorimaea operculella*; where also Ecotech bio and MVP_{11} were more effective than Dipel 2x against the second and fourth larval instars of *S. littoralis*, El-Khawas (2000) on the olive leaf moth *Palpita unionalis* larvae by using the bioinsecticide Xentari. Atalla *et al.,(*2001) on the three insect pests, *S. littoralis*, the black cutworm *Agrotis ipsilon* and corn stalk borer *S. cretica* when evaluating the effect of Agerin bioinsecticide.

Regarding the LT_{50} values (Tables 3) and (Figs. 7) a negative relationship could be detected between the applied concentration of Dipel and LT_{50} value; i.e. the LT_{50} was shortened with the increase in Dipel concentration in larvae pests. The LT_{50} values being 14.7, 8.81,5.99 and 2.40 days for the used concentrations of 8, 12, 16 and 20 × 10^4 I.U. These results are in agreement with those of Moawad *et al.,* (1982 / 1983) tested Bactospine and Diple powders on larvae of *Earias insulana;* Kares *et al.,* (1992) studied the efficacy of Bactospine on *A. rapae* **l**arvae and Kares *et al.,* (2002) tested the bioinsecticide Delfin against larvae of *O. nubilalis*.

II- Bioinsecticide (Dipel 2x) + 05 % amino acid

The larval daily mortality of *S. littoralis* treated with different concentrations of Dipel + 05 % amino are shown in Table (1), the corrected mortality percentages after 3 days increased by increasing concentration and ranged from 40.00 to 90.00 % at the concentrations of 8 to 28 × 10^4 I.U. Whereas, as shown in Fig. (2), the LC_{50} value was 11.31 × 10^4 I.U. for *S. littoralis* after 3 days.

Also by regarding the LT_{50} values (Table 3 and Fig. 8) a negative relationship could be detected between the applied concentration of Dipel + 0.5 % amino acid and LT_{50} value; i.e. the LT_{50} was shortened with the increase in treatments concentration. These values were 6.66, 3.88 and 2.18 days for the used concentrations of 8, 12 and 16 × 10^4 I.U. , respectively (Table 3).

By comparing the effects of the two treatments (Dipel and Dipel + 0.5% amino) on the mortality percentages at same concentration used, Diple + 0.5 % treatment led to increase the percentages of mortality in all concentrations used and thus led to decrease the value of LC_{50} from 17.14 to 11.31 × 10^4 I.U. and shortened also the values of LT_{50} from 14.7, 8.81 and 5.99 to 6.66,3.88 and 2.18 days at concentration of 8, 12 and 16 × 10^4 I.U. , respectively.

Table (1): Corrected mortality percentages for second instar larvae of *S. littoralis* fed on caster bean treated with Dipel 2x and its mixtures .

Treatments	Concentration I . U.	% cumulative mortality after days of treatments						
		3	8	12	16	23	26	30.00
Dipel	0	0.00	0.00	3.33	3.33	6.67	6.67	10.00
	8×10^4	36.67	43.33	43.33	46.67	53.33	50.00	63.33
	12×10^4	40.00	40.67	46.67	53.33	63.33	66.67	73.33
	16×10^4	46.67	50.00	53.33	56.67	70.00	73.33	76.67
	20×10^4	53.33	63.33	70.00	70.00	73.33	76.67	8.000
	24×10^4	56.67	66.67	73.33	76.67	76.67	76.67	83.00
	28×10^4	63.33	66.67	73.33	76.67	80.00	83.33	83.33
Dipel +0.5 % amino	0	1.67	3.33	6.67	6.67	10.00	10.00	10.00
	8×10^4	40.00	53.33	56.67	60.00	63.33	70.00	73.33
	12×10^4	50.00	56.67	60.00	63.33	66.67	73.33	76.67
	16×10^4	56.67	60	63.33	66.67	70.00	73.33	80.00
	20×10^4	76.67	76.67	80.00	83.33	86.67	90.00	93.33
	24×10^4	83.33	86.67	90.00	93.33	96.67	100	
	28×10^4	90	93.33	96.67	100			
Dipel + 1 %amino	0	1.67	3.33	6.67	6.67	10.00	10.00	10.00
	8×10^4	43.33	43.33	45.67	50.00	53.33	60.00	63.33
	12×10^4	43.33	43.33	50.00	56.67	63.33	70.00	73.33
	16×10^4	50.00	53.33	63.33	66.67	73.33	73.33	76.67
	20×10^4	53.33	56.67	66.67	70.00	73.33	76.67	80.00
	24×10^4	56.67	63.33	73.33	73.33	73.33	76.67	80.00
	28×10^4	63.33	66.67	70.00	73.00	76.67	80.00	83.33
Dipel + 1.5 %amino	0	0.00	0.00	3.33	3.33	6.67	6.67	10.00
	8×10^4	30.00	40.00	50.00	53.33	56.67	56.67	60.00
	12×10^4	36.67	50.00	63.33	66.67	66.67	70.00	70.00
	16×10^4	50.00	60.00	66.67	70.00	73.33	73.33	76.67
	20×10^4	56.67	66.67	70.00	73.33	76.67	76.67	80.00
	24×10^4	63.33	70.00	73.33	76.67	80.00	83.33	83.33
	28×10^4	70.00	73.33	76.67	80.00	83.33	83.33	86.67
Dipel + 2.5 %amino	0	0.00	0.00	3.33	3.33	6.67	6.67	10.00
	8×10^4	26.67	43.33	50.00	53.33	66.67	66.67	70.00
	12×10^4	43.33	50.00	53.33	60.00	66.67	70.00	73.33
	16×10^4	50.00	53.33	60.00	66.67	70.00	73.33	76.67
	20×10^4	53.33	60.00	66.67	70.00	73.33	76.67	80.00
	24×10^4		66.67	70.00	73.33	76.67	80.00	83.33
	28×10^4	66.67	70.00	73.33	76.67	80.00	83.33	90.00

III Diple2x + 1% Amino Acid

The daily corrected mortality percentages resulting from the treatment of 2nd instar larvae of *S. littoralis* are shown in Table (1). Larval mortality percentages, 30 days post treatment were 63.00, 73.33, 76.67, 80.00 , 80.00 and 83.33 % at concentrations of 8, 12, 16, 20, 24 and 28×10^4 I.U., respectively. The percent mortality percentages after 72 hours of treatment (estimated LC_{50}) ranged from 43.33 to 63.33 % at concentrations of 8 to 28 ×

10^4 I.U . it is evident from Table (1) that the percent mortality increased as result of increasing the concentration. The LC_{50} value was 15.59× 10^4 I.U. 72 hours post treatment (Table, 2 and Fig., 3).

Also by regarding the LT_{50} values (Table 3 and Fig. 9) a negative relationship could be detected between the applied concentration of Dipel + 1% amino and LT_{50} value; i.e. the LT_{50} was shortened with the increase in treatments concentration. These values were 11.61,7.97, 3.99, 3.07 and 1.72 days for the used concentrations of 8, 12, 16, 20 and 24 × 10^4 I.U. , respectively .

Table (2): Comparative toxicity of second instar larvae of *S. littoralis* treated with different concentrations of bioinsecticide (Dipel) and amino acid

Treatments	Day after treatments	LC 50	Slope	Confidence limits at Po. 0.5 of LC 50
Dipel 2x	3	17.14 × 10^4	1.42± 0.2845	14.39 × 10^4 : 20.63 × 10^4
Dipel 2x +0.5 % amino	3	11.31× 10^4	2.81 ±03058	9.90× 10^4: 12.52× 10^4
Dipel2x + 1 % amino	3	15.59× 10^4	1.91 ±0.2893	13.58× 10^4 : 17.67 × 10^4
Dipel 2x + 1.5 % amino	3	16.06× 10^4	1.97±0.5302	12.21× 10^4 :20.52 × 10^4
Dipel 2x + 2.5 % amino	3	16.62× 10^4	1.78± 0.5265	12.33× 10^4 :22.32 × 10^4
Amino	3	11.20%	1.57 ± 0.3502	6.34 % : 45.92 %

V Diple 2x + 1.5 % amino acid

After 3 days of treatment, the corrected mortality percentages were 30.00, 36.67, 50.00, 56.67,63.33 and 70.00 % at concentration of 8, 12, 16, 20, 24 and 28× 10^4 I.U ,respectively (Table, 1). The LC50 value was 16.06 × 10^4 I.U. (Table, 2 and Fig.,4).

LT_{50} values (Table, 3 and Fig.,10) indicated a negative relationship between the applied concentrations of Dipel + 1.5 % amino and LT_{50} values. These values were 14.12, 6.73, 3.13 and 1.69 days at concentrations of 8,12,16 and 20× 10^4 I.U. , respectively. The same results were recorded by Ebaid (2001), when carrying out laboratory studies to evaluate the effect of Consult on larvae of *S. cretica*; El-Khawas (2000) when evaluating the effect of Consult on *A. ipsilon* larvae and Mansour (2001) who estimated the effect of Mimic on *S. littoralis* larvae.

VI Diple 2x+ 2.5 % amino acid

After3 days from treatment, the mortality percentages ranged from 26.67 to 66.67 % by using concentration ranging from 8 to 28 × 10^4 I.U., respectively. Data presented in Table (1) revealed that the mortality percentages increased by increasing the tested concentrations of the bioinsecticide Dipel. However, as shown in Table (2) and Fig. (5) **,** the LC_{50} value was 16.62 × 10^4 I.U.. while the LT_{50} values were 11.18, 6.56, 4.15 and 2.80 days at concentrations of 8, 12, 16 and 20 × 10^4 I.U., respectively (Table, 3 and Fig., 11). These values indicated a negative relationship between applied concentrations of Dipel and LT_{50} values.

Results agreed with finding of Badawy (2000) when he tested Dipel 2x, Ecotech bio and MVP_{11} against *S. littoralis* and the potato tuber moth *P. operculella* where also Ecotech bio and MVP_{11} were more effective than Dipel 2x against the second and fourth instar larval of *S. littoralis* ; El-Khawas (2000) on the olive leaf moth *Palpita unionalis* larvae by using the bioinsecticide Xentari; Atalla *et al*., (2001) on the three insect pests, *S. littoralis , Agrotis ipsilon and Sesamia cretica*

when evaluating the effect of Agerin bioinsecticide; Ebaid (2001) on larvae of *O. nubilalis* by studied the effect of Delfin bioinsecticide.

Table (3): Comparative mortality time values of second instar *S. littoralis* larvae fed on caster bean treated with different concentrations of Dipel and amino acid.

Treatments	concentration	LT_{50} days	Slope	Confidence limits at Po 0.5 of LT_{50}
Dipel 2x	8×10^4	14.7	0.64±0.1488	9.78 :20.92
	12×10^4	8.81	0.86± 0.1498	6.08 : 11.42
	16×10^4	5.99	0.808± 0.149	3.42 : 8.25
	20×10^4	2.4	0.696± 0.151	0.64 :4.26
Dipel2x + 0.5% amino	8×10^4	6.66	0.8028 ± 0.1491	3.97 : 9.05
	12×10^4	3.88	0.6647± 0.1488	1.36 :6.19
	16×10^4	2.18	0.5666 ± 0.1496	0.30 :4.35
Dipel2x + 1 % amino	8×10^4	11.61	1.91± 0.2893	6.08 : 18.77
	12×10^4	7.97	0.49 ± 0.1472	5.13 : 10.59
	16×10^4	3.99	0.80 ± 0.1491	1.73 : 6.05
	20×10^4	3.07	0.76 ± 1496	1.13 : 4.97
	24×10^4	1.72	0.63 ± 0.1518	0.26 : 3.51
Dipel2x + 1.5% amino	8×10^4	14.12	0.78 ± 0.1505	10.52 : 19.12
	12×10^4	6.73	0.90 ± 0.1500	4.35 : 8.88
	16×10^4	3.13	0.71± 0.1501	1.07 : 5.13
	20×10^4	1.69	0.64 ± 0.1521	0.27 : 3.44
Dipel 2x + 2.5 % amino	8×10^4	11.18	1.15 ± 0.1544	8.97 : 13.53
	12×10^4	6.56	0.79 ± 0.1489	3.82 : 8.98
	16×10^4	4.15	0.73 ± 0.1492	1.76 : 6.32
	20×10^4	2.80	0.73± 0.1507	0.91 : 4.69

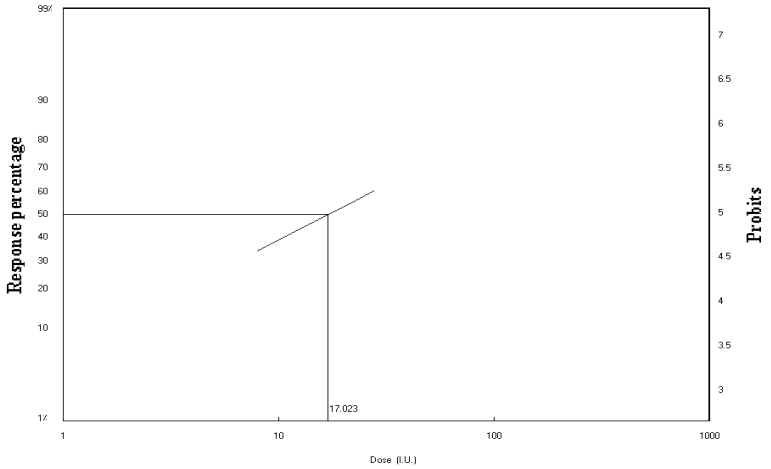

Fig. (1): Log concentration probit lines showing response of second instar larvae of *S. littoralis* treated with Dipel 2x (computed from 72 hours mortality data.

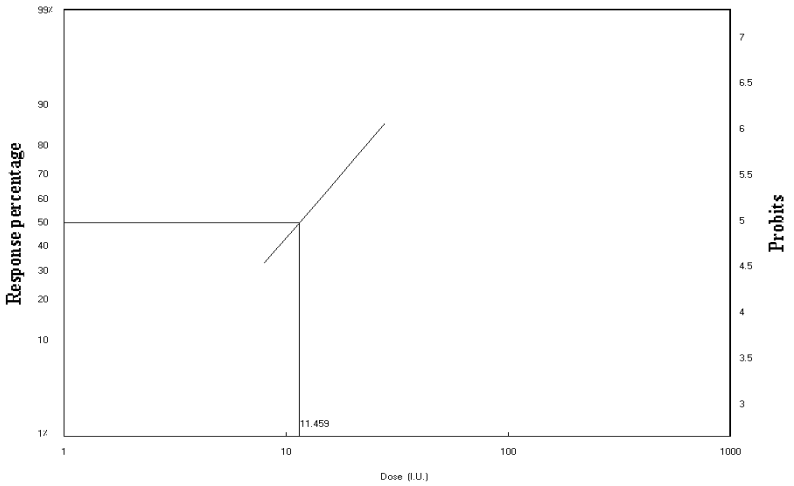

Fig. (2): Log concentration probit lines showing response of second instar larvae of *S. littoralis* treated with Dipel 2x + 0.5 % amino (computed from 72 hours mortality data.

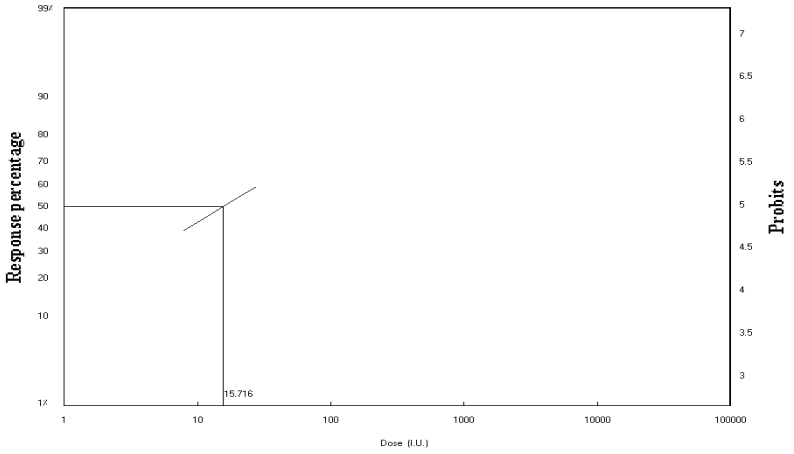

Fig. (3): Log concentration probit lines showing response of second instar larvae of *S. littoralis* treated with Dipel 2x + 1 % amino (computed from 72 hours mortality data.

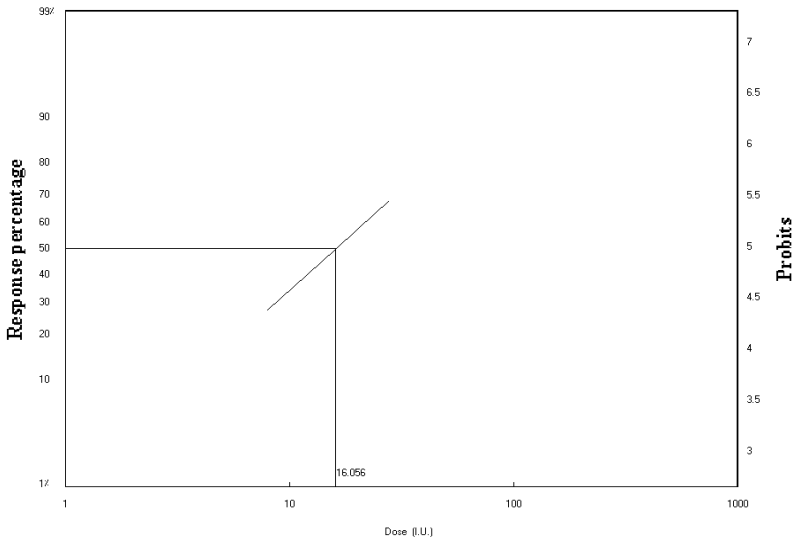

Fig. (4): Log concentration probit lines showing response of second instar larvae of *S. littoralis* treated with Diple 2x +1.5 amino (computed from 72 hours mortality data.

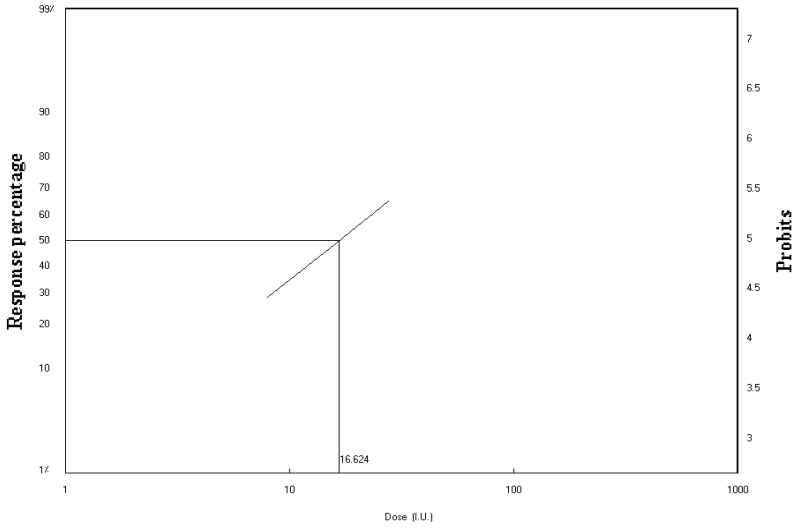

Fig. (5): Log concentration probit lines showing response of second instar larvae of *S. littoralis* treated with Diple2x + 2.5 % amino (computed from 72 hours mortality data.

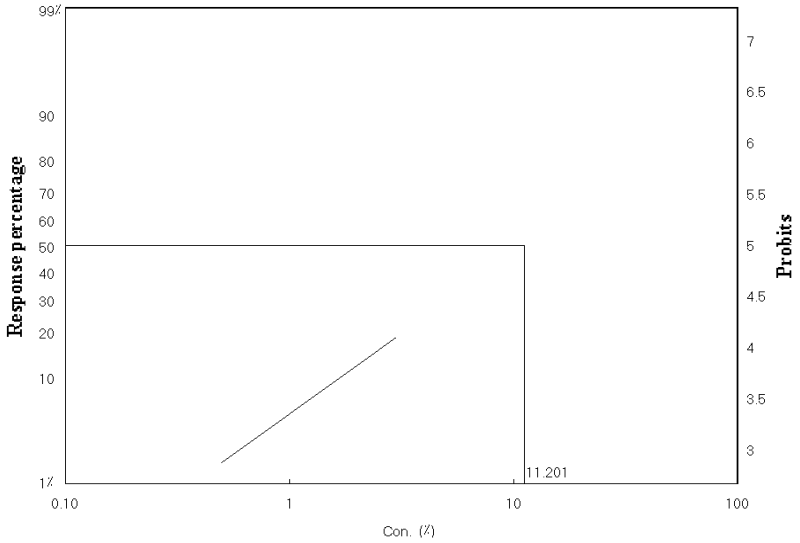

Fig. (6): Log concentration probit lines showing response of second instar larvae of *S. littoralis* treated with different concentration of amino acid.

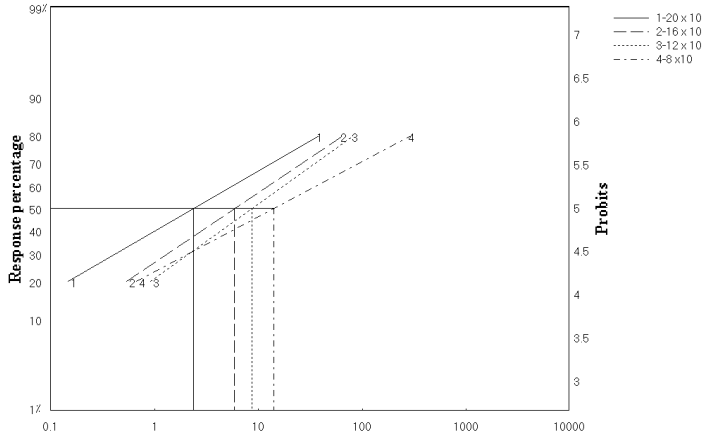

Fig. (7): Probit regression mortality time showing response of 2rd instar S. littoralis larvae at concentration of 8, 12,16 and 20× 104 I.U of Diple

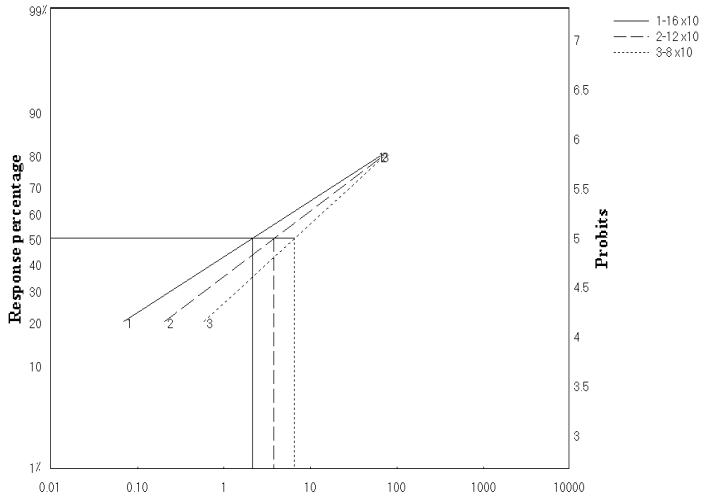

Fig. (8): Probit regression mortality time showing response of 2rd instar S. littoralis larvae a concentration of 8, 12 and 16× 104 I.U of Diple2x + 0.5 % amino t

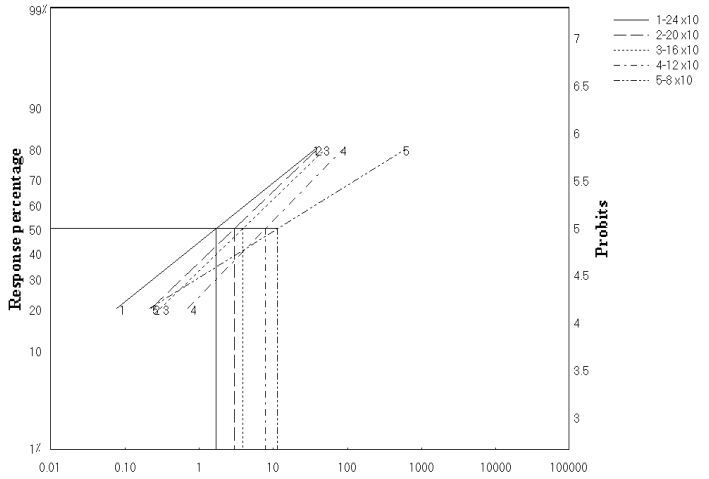

Fig. (9): Probit regression mortality time showing response of 2rd instar S. littoralis larvae at concentration of 8, 12, 16, 20 and 24 × 104 I.U of Diple + 1 % amino

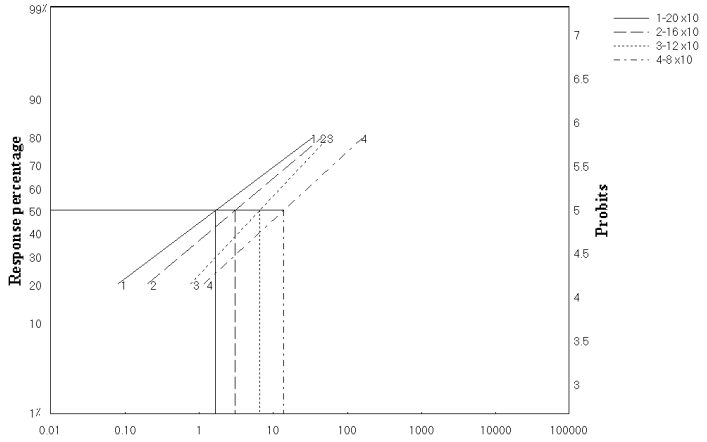

Fig. (10): Probit regression mortality time showing response of 2rd instar S. littoralis larvae at concentration of 8, 12, 16 and 20× 104 I.U of Diple + 1.5 % amino

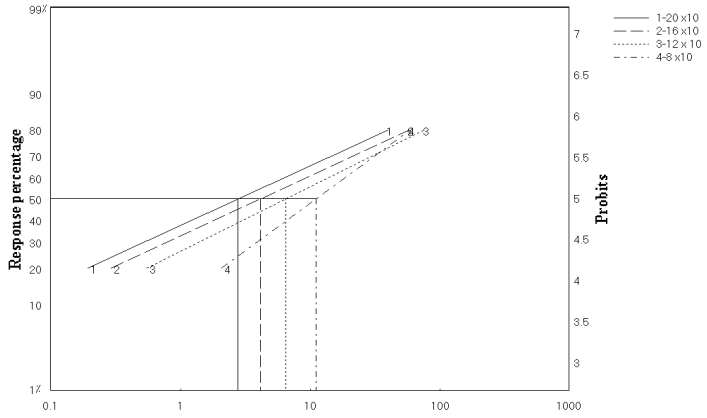

Fig. (11): Probit regression mortality time showing response of 2rd instar S. littoralis larvae at concentration of 8, 12, 16 and 20× 104 I.U of Diple + 2.5 % amino

CONCLUSION

By comparing the effects of different treatments on the LC_{50} values, data presented in Table (2) revealed that, LC_{50} value of Dipel 2x + 0.5 % amino (11.31×10^4 I.U.) was the most effective one among all treatments. Also, these treatment led to shortened the values of LT_{50} (6.66, 3.88 and 2.18

days for the used concentrations of 8, 12 and 16 × 10^4 I.U. , respectively) in comparison to other treatments (Table 3). The bioinsecticide Dipel 2x could be recommended against *S. littoralis* which causes sever damage to wide variety of Egyptian crops. The bioinsecticide will be surely not harmful to the beneficial naturals, enemies especially parasitoids and predators, will also be safe to farm animals, and help in minimizing the environmental pollution by chemical insecticides.

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تقييم فعالية *Bacillus thuringiensis kurstaki* منفردا أو مخلوطا بأحد الأحماض الأمينيه في مكافحة دودة ورق القطن

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أجريت هذه الدراسه بهدف تقييم فعالية *Bacillus thuringiensis kurstaki* منفردا أومخلوطا بأحد الأحماض الأمينيه ضد دودة ورق القطن غذيت يرقات العمر الثاني من دودة ورق القطن علي أوراق من الخروع معاملة بتركيزات مختلفة من المبيد الحيوي البكتيري الد يبيل وخليط من المبيد البكتيري مع تركيزات مختلفه من الحمض الأميني بنسبة ٠.٥ -١-١.٥-٢-٢.٥% وكانت فترة التغذيه ٤٨ ساعه .

حسبت قيمة التركيز القاتل ل ٥٠% من اليرقات المعامله والتي حسبت بعد ٧٢ ساعه من المعامله فكانت ١٧.١٤×١٠[١٤] وحده دوليه للمبيد البكتيري منفردا .

بينما في معاملة المبيد البكتيري +٠.٥ % حمض اميني وجد أن قيمة التركيز القاتل ل٥٠% قد إنخفضت ١١.٣١×١٠[١٤] وحدة دولية في حين أن هذة القيمه قد سجلت إرتفاعا مره ثانيه في المعاملات الثلاثة الخاصة بالمبيد البكتيري +٠.٥،١.٥،٢.٥ % حمض اميني لتسجل ١٥.٥٩ ،١٦.٦ ،١٦.٦٢ ×١٠[١٤] وحدة دوليه علي الترتيب بصفة عامه لوحظ وجود علاقه طرديه بين نسب الموت المسجله والتركيزات المستخدمه من المبيد

أيضا درس الوقت اللازم لموت ٥٠% من اليرقات المعاملة في المعاملات المختلفه حيث تلاحظ وجود علاقة عكسيه بين التركيزات المستخدمه من المبيد والوقت اللازم لموت ٥٠% من اليرقات .

أوضحت النتائج أن معاملة المبيد الحيوي +٠.٥% حمض أميني كانت أقل في الوقت اللازم لموت ٥٠% من اليرقات مقارنة بباقي المعاملات سجلت ٦.٦٦ ،٣.٨٨ ،٢.١٨ يوم عند التركيزات ٨،١٢،١٦ ×١٠[١٤] وحدة دوليه.