activation of *bacillus thuringiensis* var. *kurstaki* by using some chemical compounds for increasing its efficacy

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ABSTRACT

Second instar larvae of *Spodoptera. littoralis* were fed on castor bean leaves treated with different concentrations of Dipel (*Bacillus thuringiensis* var. *kurstaki*), Helban (chemical insecticide) and combination of Dipel with LC_{10} of Helban, 1% sodium chloride (NaCl) or Dipel with 1 % sodium bicarbonate($NaHCO_3$). Larvae fed for 24 hours on treated castor bean leaves dipped for one minute at each concentration used of the tested materials. The LC_{50} value of Helban was 10.26 p.p.m (estimated after 24h.from treatment) ,while the LC_{10} was 5.7 p.p.m., bioinsecticide treatment proved to be effective against 2nd instar larvae of *S. littoralis* where, the recorded LC_{50} value was15.2×10^4 I.U after 5 days of treatment. While the LT_{50} value was 4.78 and 2.75 days at concentrations of 16 and 20×10^4 I.U., respectively. In case of Dipel + 1 % $NaHCO_3$, this treatment did not offer effect more than Dipel treatment only, where the estimated LC_{50} value was 14.92×10^4 I.U. after 5 days of treatment. While the LT_{50} values were 4.78 and 2.03 days at concentrations of 16 and 20×10^4 I.U., respectively. The most effective toxic treatment among all treatments were that of Dipel + 1% NaCl where the estimated LC_{50} value of this treatment was 5.39×10^4 I.U. 5 days post treatment , While the LT_{50} values were 4.831, 3.081 and 2.096 days at concentrations of 4,8 and 12×10^4 I.U., respectively. To evaluate the joint action of the bioinsecticide Dipel combined with LC_{10} of Helban two methods were followed, the first by determining the LC_{50} values and the second by estimating the Co-toxicity factor. The LC_{50} values were 6.5×10^4 I.U. + 5.7 p.p.m. The low concentrations of Dipel when mixed with LC_{10} of Helban produced potentiation, while the high concentrations produced additional effects.

Key words: *Bacillus thuringiensis* var. *kurstaki*, *Spodoptera. Littoralis*, chemical insecticide, sodium bicarbonate, sodium chloride.

INTRODUCTION

Over the last half-century, the intensive use of synthetic organic pesticides, although useful at controlling various pests, has not been without problems. 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 *Bacillus 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).

MATERIALS NAD METHODS

1-Rearing of *S. littoralis*

A laboratory stock culture of *S. littoralis* started with larvae collected from the field and reared at 27 ±3 °C & 65 ± 5 % R.H. according to the methods recorded by Mansour (2001) for rearing *S. littoralis* on castor-bean leaves.

2-Materials used:

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

b- The chemical insecticide: Helban 48 % E. C., -O,O-diethyl O-3,5,6 trichloro-2-pyridyl phosphorothioate.

3- Treatments:

Five experimental treatments were carried out as follows:

A- Bioinsecticidal treatments:

Weights of 1.25, 2.5, 3.75, 5.00 and 6.25 gm. of Dipel were diluted in water to obtain a constant volume of 200 ml (total volume), to represent the concentrations of 4, 8 12, 16 and 20 × 10^4 I.U., respectively.

B- Chemical insecticide treatments:

A volume of 2 ml. of Helban 48 % E. C. was diluted in water to obtain constant volume of 200 ml. (total volume), to give the stock solution of 4800 p.p.m. five volumes of 0.2, 0.4, 0.62, 0.84 and 1.4 ml. of the stock solution were diluted in water to obtain a constant volume of 200 ml., representing the five concentrations of 5, 10, 15, 20, and 25 p.p.m.., respectively.

C- Combination treatments:

1- Five concentrations of 4, 8, 12, 16 and 20 × 10^4 I.U. of Dipel were prepared as previously described and mixed with LC_{10} of Helban (calculated from scale drawing after 24 hours from treatment).

2- Five concentrations of 4, 8, 12, 16 and 20 × 10^4 I.U. of Dipel were prepared as previously described and mixed with 2 gm. of sodium bicarbonate (1%).

3- Five concentrations of 4, 8, 12, 16 and 20 × 10^4 I.U. of Dipel were prepared as previously described and mixed with 2 gm. of sodium chloride (1%).

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.

The following procedures were applied:

1- 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 castor bean leaves treated with the bioinsecticide or with chemical insecticide and combination of bioinsecticide with LC_{10} of chemical insecticide or with sodium bicarbonate, and sodium chloride

2- Mortality rates were recorded daily. Larvae that survived after treatment were transferred to other jars containing untreated castor bean leaves.

3- Before exposing the larvae to treated food, they were starved for 4 hours in order to obtain rapid simultaneous ingestion of the contaminated food.

4- Control test was conducted by dipping clean castor bean leaves in water, left to dry and then offered to the experimental larvae.

5- The experiments were carried out under laboratory conditions of 27 ± 3 °C and 65 ± 5 % R.H.

Statistical analysis:

1- As larval mortality percentages in control treatments, ranged from zero to 5 % accordingly no correction on the obtained mortalities from treatments was followed.

2- 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.

3- Combination treatments: The combined action of the chemical mixture was expressed as the Co-toxicity factor estimated according to the equation of Sun and Johnson (1960) who introduced simple method for the calculation of joint toxicity of various insecticide mixtures.

$$\text{Co-toxicity factor} = \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}} \times 100$$

This factor was used to differentiate the results into three categories. Positive factor of 20 or more meant potentiation, negative factor of 20 or more meant antagonism, and any intermediate value (i.e. between -20 and + 20) was considered as additive effect.

RESULTS AND DISCUSSION

1-Toxic effect of chemical insecticide Helban on *S. littoralis*:

As shown in Table (1), mortality percentages after 24 hours for 2nd instar larvae of *S. littoralis* larvae treated with chemical insecticide Helban were 10.00, 36.67, 80.00, 96.67 and 100 % by using concentrations of 5, 10, 15, 20 and 25 p.p.m., respectively. The LC_{50} value was 10.26 p.p.m., while the LC_{10} was 5.7 p.p.m. (Table 2 and Fig.1 –a).

2-Effect of tested biocide; Dipel on second instar larvae of *S. littoralis*.

Daily mortalities among treated second instar *S. littoralis* larvae are shown in Table (1), the corrected mortality percentages after five days of treatment increased by increasing Dipel concentrations and ranged from 16.67 to 76.67 % at the concentrations of 4 to 20 × 10^4 I.U. as shown in(Table, 1 and Fig., 1-b), the LC_{50} value was 15.2 × 10^4 I.U

The increased mortality percentages by increasing the concentrations of Dipel 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.

Data of LT_{50} values indicated a negative relationship could be detected between the applied concentrations of Dipel and LT_{50} value. These values were 4.78 and 2.75 days for the used concentrations 16 and 20 × 10^4 I.U.**,** respectively, (Table, 3 and Fig., 2). These results are in agreement with those of Moawad *et al.,* (1982 / 1983) who tested Bactospine and Diple powders on larvae of *Earias insulana;* Kares *et al.,* (1992) who studied the efficacy of Bactospine on *Artogeia rapae* larvae and Kares *et al.,* (2002) who tested the bioinsecticide Delfin against larvae of *O. nubilalis*.

3-Combination treatments:

a- Effect of Dipel + 1 % sodium bicarbonate (NaHCO3):

After 5 days of treatment, the mortality percentages were 16.67, 30, 33.33, 40 and 76.67 % at concentrations of 4, 8, 12, 16 and 20 × 10^4 I.U. ,respectively (Table, 1). The LC_{50} value was14.92 × 10^4 I.U (Table, 2 and Fig., 1-c).

LT_{50} values (Table, 3 and Fig., 3) indicated a negative relationship between the applied concentrations and LT_{50} values. These values were 4.78 and 2.03 days at concentrations of 16 and 20 × 10^4 I.U., respectively.

b- Effect of Dipel +1 % sodium chloride (Nacl) on *S. littoralis* :

The second instar larvae of *S. littoralis* were fed on castor bean leaves treated with different concentrations of Dipel + 1 % NaCl. Daily mortalities among treated larvae are shown in Table (1), mortality percentages after 5 days of treatment increased by increasing Dipel concentrations and ranged from 43.33 to 90 % at the concentrations of4to20× 10^4 I.U.

The LC_{50} value was 5.39 × 10^4 I.U. with confidence limits at p< 0.05 (4.19× 10^4: 6.42 × 10^4) for *S. littoralis* after 5 days of treatment (Table, 2 and Fig., 1-d). The slope values of LC-p lines (1.95 ± 0.2482) indicated that *S. littoralis* larvae responded homogeneously to the tested bioinsecticide, whereas the deviation from parallelism was not significant for the line. The LT_{50} values were 4.831, 3.081 and 2.096 days by using the concentrations of 4, 8, and 12 ×10^4 I.U. (Table, 3 & Fig., 4)

A negative relationship could be detected between the applied concentrations of Dipel and LT_{50} value; i.e. the LT_{50} was shortened by increasing Dipel concentrations.

Makkar and El-Mandarawy, (1996) indicated that the addition of 1 % pure NaCl to the commercial product of *Bacillus thuringiensis* (Delfin) gave better mortalities results than by adding glucose. Hafez *et al.,* (2003)indicated that ,spraying maize plant with Delfin + 1% NaCl after 20 days from sowing led to 66.51 and 38.8 % reduction in the numbers of perforated leaves due to *Sesamia cretica* feeding throughout 1998 and 1999 season. Morris *et. al.,* (1996) indicated that the addition of 0.5 % wt./vol. sodium chloride and 0.1

% vol./vol. Tween 60 to a culture medium containing cotton seed meal and glucose as the main nitrogen and carbohydrate sources, respectively increased the potency of the spore-crystal product. Ghribi *et al.,* (2005), indicated that the addition of NaCl to media containing *Bacillus thuringiensis* cell led to improved delta-endotoxin production by increasing the spore titers without significant effect on toxin synthesis yields.

By comparing the effect of the two additive matrials (NaCl and $NaHCO_3$) with that of Dipel only, on the mortality percentages after 5 days of treatment at which LC_{50} caculated. The recorded values for Dipel +1% NaCl were 43, 60, 70, 83.33 and 90 % with LC_{50} value of 5.39 $\times 10^4$ I.U. for Dipel + 1% $NaHCO_3$ were, 16, 30, 33.33, 40 and76.67% with LC_{50} value 14.92$\times 10^4$ I.U opposed to 16.67, 30, 43.33, 50 and 60% with LC_{50} value of 15.2$\times 10^4$ I.U. these results indicated that Dipel + 1% NaCl was the most toxic effect on 2nd instar larvae of *S. littoralis* larvae. There is no difference between the two treatments of Dipel only or after addition of 1% $NaHCO_3$.

c- Effect of Dipel+ LC_{10} of chemical insecticide Helban on *S. littoralis*:

After 5 days from treatment with combination of different concentrations of Dipel and calculated LC_{50} of Helban (10.26 p.p.m.). The mortality percentages were 40.00, 53.33, 63.33, 70.00 and 80.00 % at concentrations of 4, 8, 12, 16 and 20 $\times 10^4$ I.U. of Dipel + LC_{10} of Helban. Two methods were followed to determine the combined effect of different Dipel concentrations with sublethal concentration (LC_{10}) of Helban. The first, by determining the LC_{50} values and the second by estimating the C0-toxicity factor.

First method: The LC_{50} values (Table, 2 and Fig., 1-f) were 6.5 $\times 10^4$ I.U. + 5.7 p.p.m.

Second method:

Data in Table (4) show that treatments by the combination of Dipel at low concentrations of 4 $\times 10^4$ and 8 $\times 10^4$ I.U. with Lc_{10} level of Helban caused mortality of 40 and 53.33 % and the values of Co-toxicity factor were + 52.85 and+ 35.01, respectively. These results indicated that the combinations of bio and chemical insecticides at the mentioned concentrations showed potentiation on their effect on larvae. While, by using the higher concentrations of Dipel (12, 16 and 20 $\times 10^4$ I.U.) combined with the LC_{10} of Helban mortality percentages were 63.33, 70.00 and 80.00, respectively. The values of c0-toxicity factor were + 19.9, + 17.64 and + 15.11, respectively indicating that these three concentrations produced additive effects.

Generally, the low concentrations of Dipel, when mixed with LC_{10} of Helban produced potentiation, while the high concentrations produced additional effects.

These results agree with El-Zemaity and El-Refai (1987) who revealed potentiation of the combination of Fenvalerate at LC_{25} and Dipel (*B. thuringiensis* subsp *kurstaki*) against larvae of *S. littoralis*. Raising the LC value of Fenvalerate revealed an additive effect. The co-toxicity factor decreased when the LC values of Fenvalerate or Dipel were increased. Mansour (2001) indicated that, the combination of the bioinsecticide (Xentari) with LC_{10} of the chemical insecticide (Baythroid caused higher mortality for unparasitized *S. littoralis* larvae than those parasitized by *M.rufiventris*. the low concentrations of Xentari, when mixed with LC_{10} of Baythroid produced additional effect in both cases of unparasitized and parasitized larvae. El-Moursy *et al.,* (2000), revealed potentiotion of the combination of bioinsecticide (Delfin) and LC_{10} level of chemical insecticide (Baythroid) against unparasitized *S. littoralis* larvae and those parasitized by *M.rufiventris*. While Delfin at higher concentrations was combined with LC_{10} level of Baythroid for unparasitized and parasitized larvae, produced additional effect.

Data of LT_{50} values indicated a negative relationship could be detected between the applied concentration of Dipel and LT_{50} value. These values were 7.087, 4.157 and 2.493 days for the used concentration 4, 8 and 12×10^4 I.U.**,** respectively, (Table, 3 and Fig., 5).

Table (1): Mortality rates for *S. littoralis* second instar larvae treated with chemical insecticide Helban, bioinsecticide Dipel and their combination.

Concentration	Cumulative mortality % after days of treatment							
	1	2	3	4	5	6	7	
	Chemical insecticide Helban							
5 p.p.m.	10.00	40.00	73.33	86.67	100.00			
10	36.67	70.00	90.00	100.00				
15	80.00	93.33	100.00					
20	96.67	100.00						
25	100.00							
0.00	0.00	0.00	3.33	3.33				
	Bioinsecticide Dipel							
4×10^4 I.U.	0.00	3.33	10.00	13.33	16.67	20.00	23.33	Survived larvae reached pupal stages
8×10^4	6.67	16.67	23.33	26.67	30.00	40.00	50.00	
12×10^4	13.33	26.67	33.33	36.67	43.33	50.00	56.67	
16×10^4	16.67	36.67	43.33	46.67	50.00	60.00	66.67	
20×10^4	20.00	46.67	53.33	56.67	60.00	73.33	83.33	
	Dipel+ 1% sodium bicarbonate							
4×10^4 I.U.	6.67	10.00	10.00	13.33	16.67	30.00	36.67	
8×10^4	10.00	13.33	16.67	23.33	30.00	43.33	50.00	
12×10^4	13.33	16.67	23.33	26.67	33.33	56.67	66.67	
16×10^4	16.67	26.67	30.00	36.67	40.00	63.33	73.33	
20×10^4	33.33	43.33	63.33	66.67	76.67	80.00	83.33	
	Dipel + 1% sodium chloride							

4×10^4 I.U.	20.00	26.67	36.67	40.00	43.33	60.00	66.67	
8×10^4	26.67	36.67	50.00	53.33	60.00	66.67	73.33	
12×10^4	33.33	50.00	56.67	63.33	70.00	73.33	80.00	
16×10^4	36.67	56.67	63.33	76.67	83.33	86.67	93.33	
20×10^4	43.33	66.67	76.67	83.33	90.00	96.67	100.00	
	Dipel + LC_{10} of chemical insecticide							
4×10^4 I.U.	16.67	26.67	33.33	36.67	40.00	46.67	53.33	
8×10^4	23.33	33.33	40.00	43.33	53.33	60.00	66.67	
12×10^4	33.33	43.33	50.00	60.00	63.33	70.00	76.67	
16×10^4	43.33	53.33	60.00	66.67	70.00	80.00	83.33	
20×10^4	46.67	60.00	66.67	73.33	80.00	86.67	93.33	

Table (2): Comparative toxicity of second instar larvae of *S. littoralis* treated with different concentrations of biocide Dipel and chemical insecticide and their mixture

Treatments	LC_{50}	Slope	Confidence limits at Po. 0.5 of LC_{50}
Chemical insecticide Helban	10.26 p.p.m.	4.99 ± 0.7573	8.84 : 11.66
Dipel	15.2×10^4 I.U.	1.73 ± 0.2536	12.95×10^4 : 18.8×10^4
Dipel + 1% sodium bicarbonate	14.92×10^4 I.U.	1.98 ± 0.2615	
Dipel + 1% sodium chloride	5.39×10^4 I.U.	1.95 ± 0.2482	4.19×10^4 : 6.42×10^4
Dipel + LC_{10} of Helban	6.5×10^4 I.U.+ 5.7 p.p.m.	1.46 ± 0.2369	4.8×10^4 : 7.9×10^4

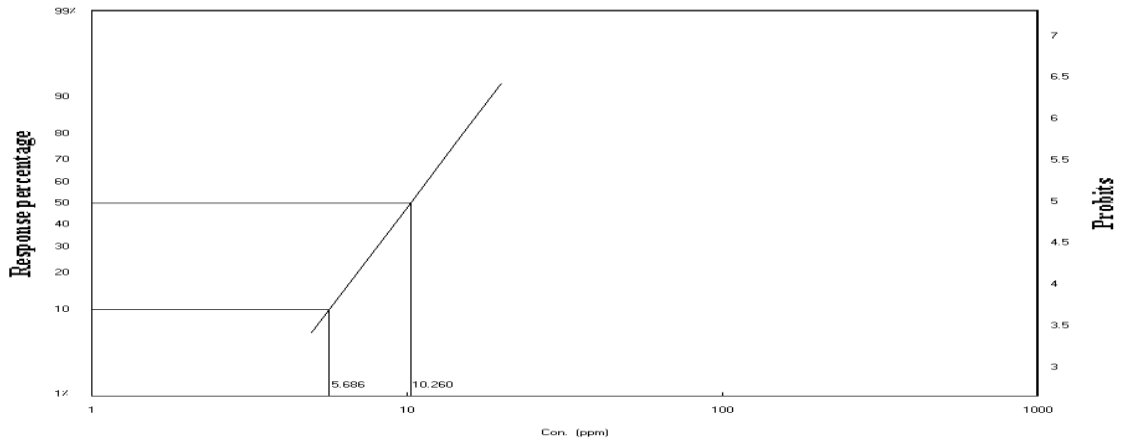
Table (3): Comparative mortality time for Dipel, Dipel+ 1% sodium bicarbonate, Dipel + 1% sodium chloride and Dipel + LC_{10} of chemical insecticide on 2nd instar larvae of *S. littoralis*.

Treatments	**Concentration**	**LT_{50} days**	**Slope**	Confidence limits at po 0.5 of LT_{50}
Dipel	16×10^4	4.78	1.74 ± 0.1991	3.564 : 8.393
	20×10^4	2.75	1.79 ± 0.1880	2.378 : 3.118
Dipel + 1% sodium bicarbonate	16×10^4	4.78	1.74 ± 0.1991	3.564 : 8.393
	20×10^4	2.03	1.72 ± 0.1858	1.675 : 2.353
Dipel + 1% sodium chloride	4×10^4	4.831	1.45± 0.1909	4.132 : 5.887
	8×10^4	3.081	1.90 ± 0.1830	2.6032 : 3.5918
	12×10^4	2.096	1.41 ± 0.1811	1.6593 : 2.4923
Dipel + LC_{10} of chemical insecticide	4×10^4	7.087	1.16 ± 0.1908	5.59 : 10.49
	8×10^4	4.157	1.33 ± 0.1849	3.52 : 5.038
	12×10^4	2.4927	1.32 ± 0.1801	2.008 : 2.56

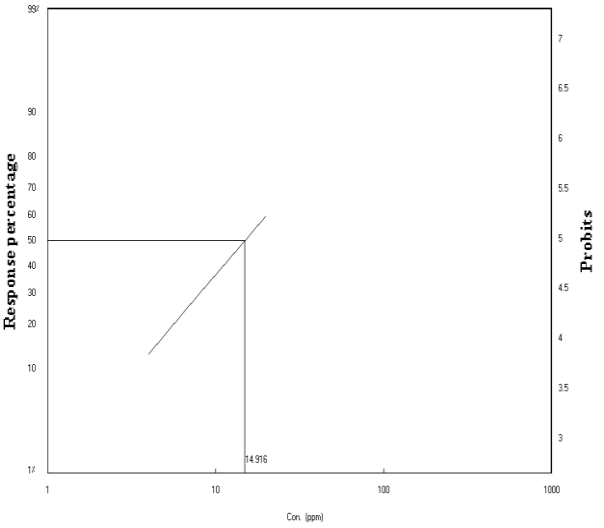
Table (4): The susceptibility of second instar larvae of *S. littoralis* to mixtures of Dipel and LC_{10} of Helban

Concentrations		Calculated % mortality		Expected % mortality	Observed % mortality	Co-toxicity factor	Combined effects
Diple I.U.	Chemical insecticide p.p.m.	Diple	Chemical insecticide				
4×10^4		16.67		26.17	40	+52.85	Potentiation
8×10^4		30		39.5	53.33	+35.01	Potentiation
12×10^4	5.7	43.33	9.5	52.83	63.33	+19.9	Addition
16×10^4		50		59.5	70	+17.64	Addition
20×10^4		60		69.5	80	+15.11	Addition

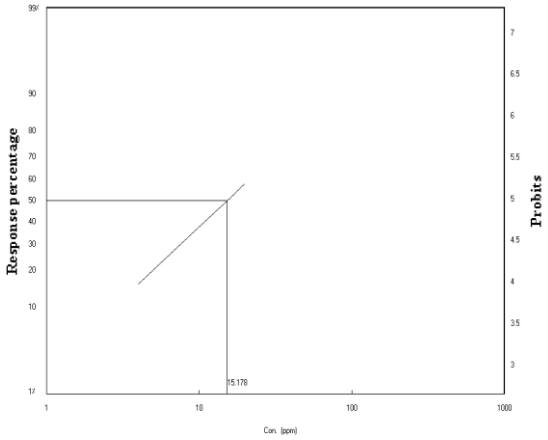
.Fig. (1): Log concentration probit line showing response of 2nd instar larvae of *S. littoralis* to different treatments



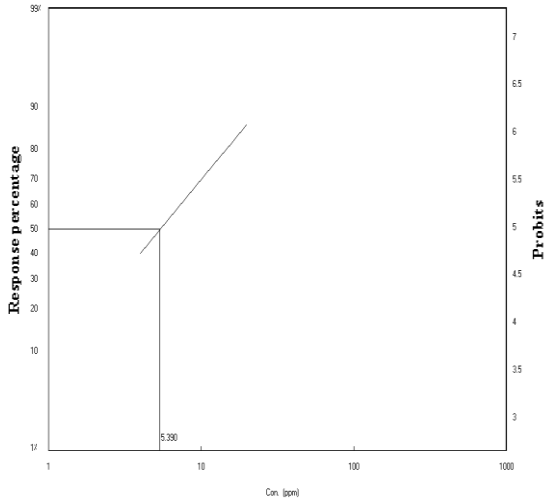
a- Chemical insecticide Helban



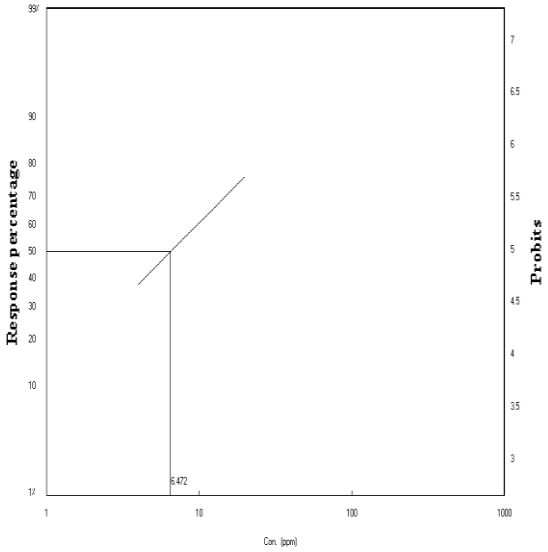
c- Dipel + 1% sodium bicarbonate



b- Bioinsecticide (Dipel



d- Dipel + 1% sodium chloride



f- Dipel + LC_{10} of Helban

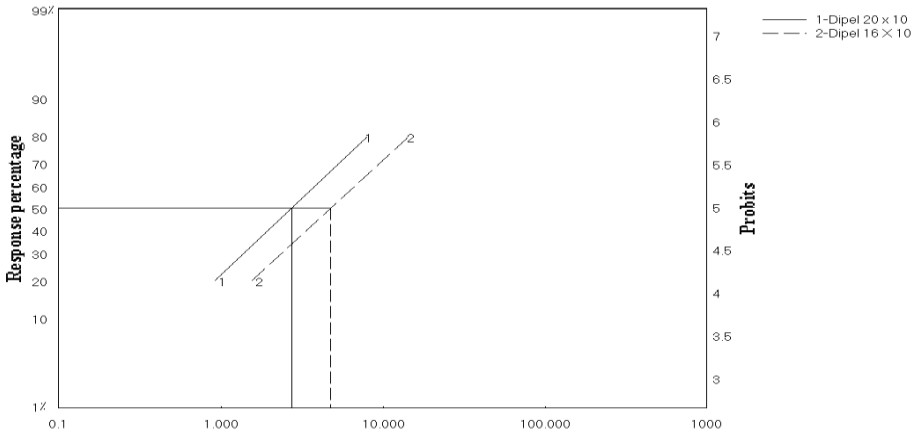


Fig. (2): Probit regression mortality time showing response of 2nd instar *S. littoralis* larvae at concentration of 16 and 20 × 10^4 I.U. of Dipel

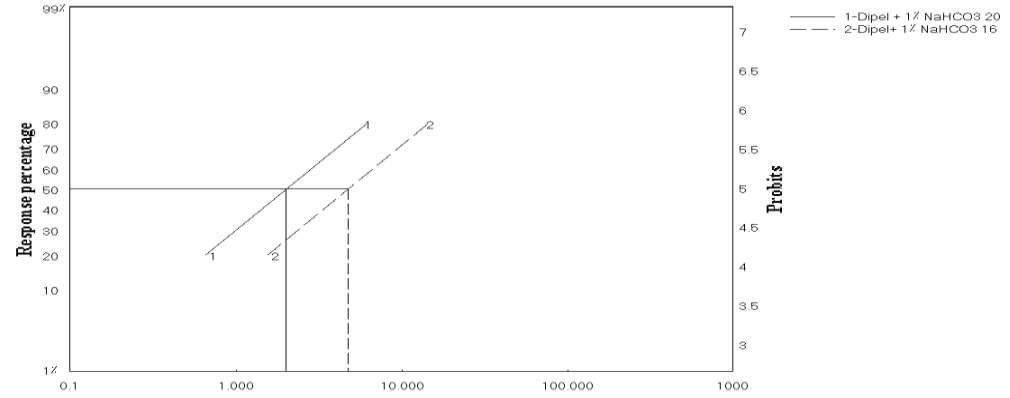


Fig. (3): Probit regression mortality time showing response of 2nd instar *S. littoralis* larvae at concentration of 16 and 20 × 10^4 I.U. of Dipel + 1% sodium bicarbonate

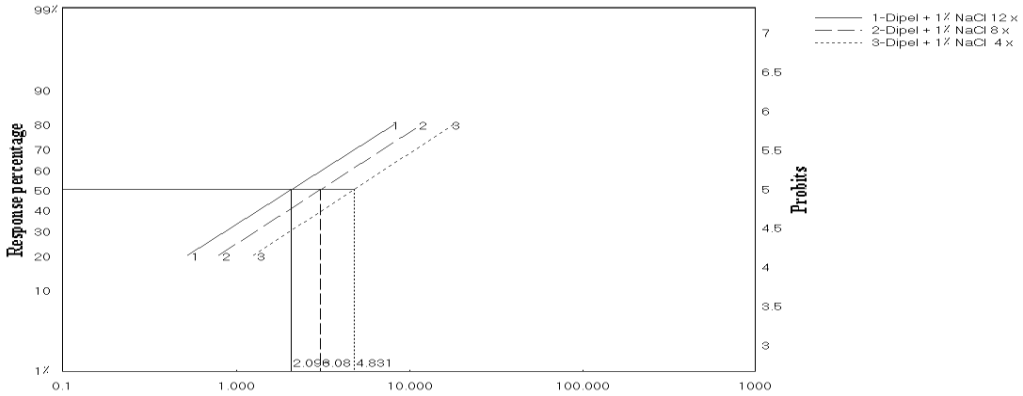


Fig. (4): Probit regression mortality time showing response of 2nd instar *S. littoralis* larvae at concentration of 4, 8 and 12 × 10^4 I.U. of Dipel + 1% sodium chloride

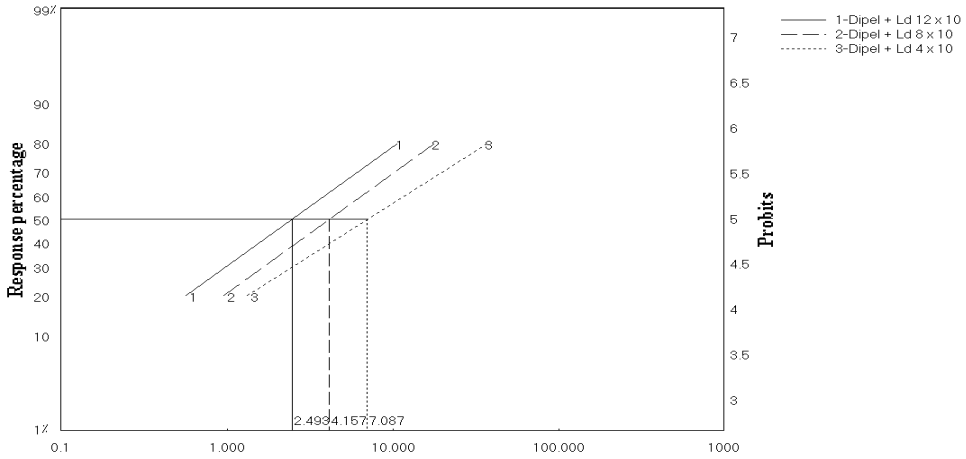


Fig. (5): Probit regression mortality time showing response of 2nd instar *S. littoralis* larvae at concentration of 16 and 20 × 10^4 I.U. of Dipel + LC_{10} of Helban

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المركبات الكيميائية المضافة إلي بكتريا باسيلس ثيورينجينسيس كورستاكي لزيادة كفائتها

عريان شحاته منصور، محمد سيد ابراهيم شلبي ،نادر أبوزيد عبده ، ساميه زين سيد

أجريت تجربه معمليه لدراسة مدي تأثير إضافة بعض المركبات الكيماوية (١% كلوريد الصوديوم أو ١%بيكربونات الصوديوم) إلي المبيد البكتيري (دايبل) مقارنة بالمبيد الكيماوي (هلبان) علي العمر الثاني لدودة ورق القطن . حيث غذيت اليرقات علي أوراق الخروع المعامل بعدة تركيزات من المبيد البكتيري والمبيد الكيماوي كلا علي حده وكذلك خليط من الدايبل مع كلا من التركيز القاتل لـ ١٠% من اليرقات لمبيد الهلبان والمركب الغير عضوي – ١% كلوريد الصوديوم وكذلك ١% بيكربونات الصوديوم . غذيت اليرقات علي الأوراق المعامله بعد غمرها لمدة دقيقه واحده لكل التركيزات المستخدمه . تم تقدير التركيز القاتل لـ ٥٠% من اليرقات لمبيد الهلبان بعد ٢٤ ساعة فكان ١٠,٢٦ جزء في المليون بينما كان التركيز القاتل لـ ١٠% هو ٥,٧% جزء في المليون. أثبتت الدراسة ان المبيد الحيوي أكثر فاعليه علي يرقات العمر الثاني لدوده ورق القطن حيث كان التركيز القاتل لـ ١٠% هو ١٥×١٠^{٤} وحده دوليه وذلك بعد ٥ أيام من المعاملة . كانت قيمة الوقت اللازم لموت ١٠% هو٤,٧٨ ، ٢,٦٥ يوم عند تركيز ١٦، ٢٠× ١٠^{٤} وحده دوليه علي التوالي . لم يكن هناك تأثير عند خلط ١% بيكربونات الصوديوم مع المبيد الحيوي مقارنة بالمبيد الحيوي منفردا حيث كان التركيز القاتل لـ١٠% هو ١٤,٢٩ × ١٠^{٤} وحده دوليه وذلك بعد ٥ أيام من المعاملة بينما كانت قيم الوقت

اللازم لقتل ٥٠% من اليرقات هي ٤,٧٨ ، ٢,٠٣ يوم عند التركيزات ١٦ ، ٢٠ × $١٠^{٤}$ وحده دوليه ، علي التوالي . أثبتت الدراسة أيضا ان خليط الدايبل + ١% كلوريد الصوديوم هو الأكثر سميه حيث كان التركيز القاتل لـ ٥٠ % من اليرقات هو ٥,٣٩ × $١٠^{٤}$ وحده دوليه وذلك لمدة ٥ أيام بعد المعاملة بينما كان الوقت اللازم لقتل ٥٠% هو ٤,٨٣١ ، ٣,٨١ ،٢,٩٦ يوم عند التركيزات ٤، ٨، ١٢ × $١٠^{٤}$ وحده دوليه، علي التوالي . اتبعت طريقتان لتقييم التأثير المشترك لمخلوط المبيد الحيوي مع ١٠% من المبيد الكيماوي .الطريقة الأولي : تقدير التركيز القاتل لـ١٠% لليرقات المعاملة

الطريقة الثانية : تقدير عامل السمية المشترك .

كان التركيز القاتل لـ ٥٠% هو ٦,٥ × $١٠^{٤}$ وحده دوليه +٥,٧ جزء في المليون .

أظهرت التركيزات المنخفضة لمخلوط المبيد الحيوي بالتركيز القاتل لـ ١٠% للمبيد الكيماوي تأثيرات تنشيطية بينما التركيزات العالية أعطت تأثيرات إضافية.